

**Beyond single biomarker-isotope
studies in paleoclimatology – potential
and limitations of a coupled
 $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer
approach**

DISSERTATION

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Johannes Hepp

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Amtierender Dekan: Prof. Dr. Stefan Peiffer

Prüfungsausschuss:

PD. Dr. Michael Zech (Gutachter)

Prof. Dr. Eva Lehndorff (Gutachterin)

Prof. Dr. Gehard Gebauer (Vorsitz)

Prof. Dr. Ludwig Zöllner

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Summary

Stable water isotopes have significantly contributed to the reconstruction of climate history qualitatively and quantitatively during the last decades. The hydrogen isotopic composition ($\delta^2\text{H}$) of terrestrial leaf wax-derived n -alkanes is used as source water $\delta^2\text{H}$ recorder, and is often interpreted as $\delta^2\text{H}_{\text{precipitation}}$. However, $\delta^2\text{H}_{n\text{-alkane}}$ is not only influenced by $\delta^2\text{H}_{\text{precipitation}}$ changes, but also by the incorporation of the leaf water enrichment signal caused by evapotranspiration. Therefore, single n -alkane $\delta^2\text{H}$ -based climate proxies are often interpreted only quantitatively. Oxygen isotopic composition ($\delta^{18}\text{O}$) of hemicellulose-derived sugars can be interpreted comparable to the $\delta^2\text{H}$ of n -alkanes. By combining $\delta^2\text{H}_{n\text{-alkane}}$ with $\delta^{18}\text{O}_{\text{sugar}}$ results, potentially a powerful tool is available for disentangling between source water and evapotranspirative enrichment changes. Such a coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach was shown to derive quantitative hydroclimate records, i.e. past $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and relative air humidity (RH_{air}) values, respectively. In previous studies, this coupling was therefore introduced as paleohygrometer approach in analogy to the often used paleothermometer approaches in Quaternary research. Within this PhD thesis I aim at contributing to the validation and the broader application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach, because a detailed validation and particularly the application to lake sediments is still missing.

The first two studies are therefore addressing the validation of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ (paleohygrometer) approach. Firstly, leaf sample material from a climate chamber experiment conducted with three different plant species was analyzed. The climate chamber experiment showed that leaf $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ are well correlated with $\delta^2\text{H}_{\text{leaf-water}}$, $\delta^{18}\text{O}_{\text{leaf-water}}$ ($r^2 = 0.45$ and 0.85 , respectively, $p < 0.001$, $n = 24$). RH_{air} was robustly reconstructed based on a simplified Craig-Gordon model. The second validation approach is a European topsoil transect study. It revealed that the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach allows the reconstruction of $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and mean RH during day-time and vegetation period (RH_{MDV}). However, systematic offsets between biomarker-based (reconstructed) $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and RH_{MDV} values and a clear larger range compared to $\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$ and climate station RH_{MDV} , respectively, were observed.

The application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach to the terrestrial climate archive Maundi (Mt. Kilimanjaro, Tanzania) was successful, allowing the reconstruction of $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and day-time RH (RH_{D}) throughout the last ~ 100 ka for East African region. The observed strong positive relationship between the biomarker-based $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and deuterium-excess of leaf water values indicates that an amount effect in precipitation isotope composition seems not to be present on long time scales.

In order to provide the backbone for applying the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach, a source identification study of terrestrial versus aquatic sugar biomarkers in lake sediments was conducted. For Late Glacial-Early Holocene sediments of Lake Gemündener Maar (Western Eifel region, Germany), the results show that arabinose is primarily of terrestrial origin while fucose and xylose stem predominantly from aquatic sources. This allows for using $\delta^{18}\text{O}_{\text{arabinose}}$ and terrestrial $\delta^2\text{H}_{n\text{-alkane}}$ results from Lake Gemündener Maar sediments in a coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach in order to derive a day-time vegetation period RH (RH_{dv}) record. The results challenge the paradigmatic view

that the Younger Dryas is characterized by dry climatic conditions. They rather suggest that a relatively wet phase at the beginning of the Younger Dryas prevailed, which is followed by a more drier ending of the Younger Dryas. Also, large RH_{dv} changes during the Early Holocene were obvious which are more pronounced than the variations during the Allerød-Younger Dryas transition phase.

Finally, I aimed to apply the coupled $\delta^2H_{n\text{-alkane}}-\delta^{18}O_{\text{sugar}}$ approach also to the Late Glacial-Early Holocene sediments of Lake Bergsee (Southern Black Forest, Germany) in order to validate or falsify the findings from Lake Gemündener Maar. However, the source identification strongly suggest that the sugar biomarkers in that lake are primarily of aquatic origin. Hence, a coupling of terrestrial $\delta^2H_{n\text{-alkane}}$ with $\delta^{18}O_{\text{sugar}}$ records was not feasible. At the same time, the coupling of aquatic $\delta^2H_{n\text{-alkane}}$ with $\delta^{18}O_{\text{sugar}}$ records was not possible either, because $n\text{-C}_{23}$ could not be considered as robust aquatic biomarker.

Overall, the findings of the studies I conducted for this thesis highlight the large potential to derive quantitative hydroclimate information from the coupled $\delta^2H_{n\text{-alkane}}-\delta^{18}O_{\text{sugar}}$ (paleohygrometer) approach. At the same time, the reconstruction of $\delta^2H_{\text{leaf-water}}$ from $\delta^2H_{n\text{-alkane}}$ turned out to be a major uncertainty, representing the limitation regarding the reconstruction of rather small variability of $\delta^2H_{\text{source-water}}$, $\delta^{18}O_{\text{source-water}}$ and RH . Furthermore, a clear differentiation between terrestrial or aquatic origins of the $n\text{-alkane}$ and sugar biomarkers seems to be of fundamental importance for a successful application of the coupled $\delta^2H_{n\text{-alkane}}-\delta^{18}O_{\text{sugar}}$ approach to lacustrine archives.

Zusammenfassung

Die Analyse der stabilen Wasserisotope hat in den letzten Dekaden maßgeblich dazu beigetragen die Klimageschichte qualitativ wie auch quantitativ zu erfassen. Zur Rekonstruktion der Isotopenzusammensetzung des Wasserstoffs ($\delta^2\text{H}$) des Wassers, welches Pflanzen aufnehmen, wird oftmals die $\delta^2\text{H}$ von n -Alkan Biomarkern aus Blattwachsen herangezogen. Diese wird wiederum als $\delta^2\text{H}$ des Niederschlags interpretiert. Allerdings werden die $\delta^2\text{H}_{n\text{-Alkan}}$ Werte nicht nur durch Veränderungen in den $\delta^2\text{H}_{\text{Niederschlag}}$ Werten beeinflusst, sondern beinhalten auch ein Anreicherungssignal, welches bei der Evapotranspiration von Blattwasser auftritt. Daher können Klimaproxies, die allein auf $\delta^2\text{H}_{n\text{-Alkan}}$ Ergebnissen basieren, oft nur qualitativ interpretiert werden. Die Interpretation der Isotopenzusammensetzung des Sauerstoffs ($\delta^{18}\text{O}$) von Hemizellulose-bürtigen Zuckern ist vergleichbar mit der Interpretation der $\delta^2\text{H}$ von n -Alkanen. Die Kopplung von Beiden erweist sich als sehr hilfreich um zwischen den Veränderungen im Wasser, welches die Pflanzen aufnehmen und Änderungen in der Evapotranspiration von Blattwasser zu unterscheiden. Solch ein gekoppelter Ansatz kann daher dazu beitragen die hydroklimatischen Bedingungen in der Vergangenheit auch quantitativ zu erfassen. Dazu werden $\delta^2\text{H}_{\text{Wasser}}$ und $\delta^{18}\text{O}_{\text{Wasser}}$ Werte und relative Luftfeuchtigkeiten (RH) rekonstruiert. In vorangegangenen Arbeiten wurde diese Kopplung als Paleohygrometer Ansatz eingeführt, in Analogie zu den in der Quartärforschung oft verwendeten Paleothermometer Ansätzen. In dieser Arbeit will Ich einen Beitrag zur Validierung und der breiteren Anwendung des gekoppelten $\delta^2\text{H}_{n\text{-Alkan}}\text{-}\delta^{18}\text{O}_{\text{Zucker}}$ Ansatzes leisten, da eine detaillierte Validierung und insbesondere eine Anwendung auf Seesedimente bislang nicht erfolgt sind.

Die ersten beiden Studien befassen sich mit der Validierung des Ansatzes. Hierfür konnte zum einen auf Blattmaterial eines Klimakammerexperimentes mit drei verschiedenen Pflanzenarten zurückgegriffen werden. Das Klimakammerexperiment zeigt, dass die $\delta^2\text{H}_{n\text{-Alkan}}$ und $\delta^{18}\text{O}_{\text{Zucker}}$ Werte gut mit den $\delta^2\text{H}_{\text{Blattwasser}}$ bzw. $\delta^{18}\text{O}_{\text{Blattwasser}}$ Werten korreliert sind ($r^2 = 0.45$ und 0.85 , $p < 0.001$, $n = 24$). Zudem konnten die RH Bedingungen der Klimakammern mit Hilfe eines simplen Craig-Gordon Modell robust rekonstruiert werden. Als zweiter Validierungsansatz wurden $\delta^2\text{H}_{n\text{-Alkan}}$ und $\delta^{18}\text{O}_{\text{Zucker}}$ in Oberbodenproben eines europäischen Transektes analysiert. $\delta^2\text{H}_{\text{Wasser}}$ und $\delta^{18}\text{O}_{\text{Wasser}}$ sowie RH Werte konnten hiermit rekonstruiert werden. Die RH Werte sind hierbei repräsentativ für die gemittelten Bedingungen während des Tages und der Vegetationsperiode (RH_{MDV}). Jedoch gibt es eine systematische Abweichung zwischen den rekonstruierten $\delta^2\text{H}_{\text{Wasser}}$, $\delta^{18}\text{O}_{\text{Wasser}}$ und RH_{MDV} Werten sowie eine größere Streuung im Vergleich zu $\delta^2\text{H}_{\text{Niederschlag}}$, $\delta^{18}\text{O}_{\text{Niederschlag}}$ und RH_{MDV} Werten der Klimastationen.

Die Anwendung des gekoppelten $\delta^2\text{H}_{n\text{-Alkan}}\text{-}\delta^{18}\text{O}_{\text{Zucker}}$ Ansatzes auf das terrestrisches Klimaarchiv Maundi (Mt. Kilimanjaro, Tansania) erlaubte die Rekonstruktion von $\delta^2\text{H}_{\text{Wasser}}$ und $\delta^{18}\text{O}_{\text{Wasser}}$ und Tageszeit RH (RH_D) während der letzten ~ 100 ka für die Region Ostafrikas. Es besteht ein starker positiver Zusammenhang zwischen den Biomarkerbasierten $\delta^2\text{H}_{\text{Wasser}}$, $\delta^{18}\text{O}_{\text{Wasser}}$ Werten und dem Deuterium-Exzess des Blattwassers. Dies deutet darauf hin, dass der Niederschlagsmengen-Effekt auf die Niederschlagsisotopie für längere Zeitskalen keinen Einfluss hat.

Um eine solide Basis für die Anwendung des gekoppelten $\delta^2\text{H}_{n\text{-Alkan}}\text{-}\delta^{18}\text{O}_{\text{Zucker}}$ Ansatzes auf Seesedimente zu schaffen, wurde zunächst untersucht, wie zwischen dem terrestrischen und aquatischen Beitrag von sedimentären Zuckerbiomarkern unterschieden werden

kann. Für die Spätglazialen-Frühholozänen Sedimente des Gemündener Maars (West-eifel, Deutschland) konnte gezeigt werden, dass Arabinose hauptsächlich terrestrischen Ursprungs ist, während Fucose und Xylose maßgeblich auf aquatischen Eintrag zurückzuführen sind. Basierend auf terrestrischen $\delta^2\text{H}_{n\text{-Alkan}}$ und $\delta^{18}\text{O}_{\text{Arabinose}}$ Ergebnissen, kann damit der gekoppelte $\delta^2\text{H}_{n\text{-Alkan}}\text{-}\delta^{18}\text{O}_{\text{Zucker}}$ paleohygrometer Ansatzes etabliert werden. Die hiermit rekonstruierten RH Werte, welche die RH Bedingungen während der Vegetations- und Tageszeit widerspiegeln (RH_{dv}), stellen die paradigmatische Vorstellung einer trockenen Jüngeren Dryas in Frage. Die Ergebnisse deuten eher darauf hin, dass die Jüngere Dryas relativ feucht begonnen hat und sich erst zum Ende dieser Periode ein trockeneres Klima einstellt hat. Zudem wird offensichtlich, dass die Schwankungen in RH_{dv} im Frühholozän im Vergleich zur Übergangsphase zwischen Allerød und Jüngeren Dryas deutlich höher sind.

Zuletzt sollte der gekoppelte $\delta^2\text{H}_{n\text{-Alkan}}\text{-}\delta^{18}\text{O}_{\text{Zucker}}$ Ansatz auf die Sedimente des Bergsees (Südschwarzwald, Deutschland) übertragen werden. Jedoch zeigte sich, dass die Zuckerbiomarker im Bergsee maßgeblich aquatischen Ursprungs sind, was eine Kopplung von terrestrischen $\delta^2\text{H}_{n\text{-Alkan}}$ mit $\delta^{18}\text{O}_{\text{Zucker}}$ Ergebnissen verhindert. Zudem war auch keine Kopplung von aquatischen $\delta^2\text{H}_{n\text{-Alkan}}$ mit $\delta^{18}\text{O}_{\text{Zucker}}$ Ergebnissen möglich, da $n\text{-C}_{23}$ nicht als robuster aquatischer Biomarker herangezogen werden kann.

Die Ergebnisse der einzelnen Studien dieser Arbeit zeigen das große Potential des gekoppelten $\delta^2\text{H}_{n\text{-Alkan}}$ mit $\delta^{18}\text{O}_{\text{Zucker}}$ Ansatzes zur Ableitung von quantitativen hydroklimatischen Informationen. Es wird jedoch auch offensichtlich, dass insbesondere die Rekonstruktion von $\delta^2\text{H}_{\text{Blattwasser}}$ basierend auf $\delta^2\text{H}_{n\text{-Alkan}}$ Werten fehlerbehaftet sein kann, was die Rekonstruktion von kleinen Schwankungen in $\delta^2\text{H}_{\text{Wasser}}$, $\delta^{18}\text{O}_{\text{Wasser}}$ und RH limitiert. Zudem ist eine deutliche Unterscheidung zwischen terrestrischen und aquatischen Ursprungs der $n\text{-Alkan}$ - und Zuckerbiomarker eine Grundbedingung für die erfolgreiche Anwendung des gekoppelten $\delta^2\text{H}_{n\text{-Alkan}}\text{-}\delta^{18}\text{O}_{\text{Zucker}}$ Ansatzes auf Seesedimentarchive.

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Extended Summary

1 Introduction

The history of human evolution, and especially its cultural history, has always been strongly linked to climate variability (e.g. Behrensmeyer, 2006; Trauth et al., 2007; Sirocko, 2012). This explains why much scientific effort is spent for a better understanding of modern climate systems and climate changes of the past. Stable isotopes have contributed significantly during the last decades to reconstructing qualitatively and quantitatively climate history. For example, the stable oxygen isotopic composition ($\delta^{18}\text{O}$) of deep-sea records serves as proxy for global ice volume and $\delta^{18}\text{O}$, as well as the hydrogen isotope composition ($\delta^2\text{H}$), of ice cores from Antarctica and Greenland are used as proxy for global temperature history (Shackleton, 1987; Petit et al., 1999; Johnsen et al., 2001). With regard to terrestrial climate archives, deriving quantitative paleoclimate information, like relative humidity (RH) and isotope composition of precipitation ($\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$), from biomarker-isotope proxy data could overcome typical limitations of so far applied qualitative interpretation approaches (Feng et al., 2007). Eley and Hren (2018) presented leaf wax-derived *n*-alkane chain-length pattern to derive past vapor pressure deficit changes. Furthermore, Gázquez et al. (2018) showed that triplicate oxygen stable isotope measurements of gypsum can be used for RH_{air} reconstructions. Triplicate oxygen isotope composition was also measured in phytoliths in order to derive RH information for a climate chamber and a topsoil transect study (Alexandre et al., 2018). Rach et al. (2017) used the differences in the hydrogen isotope composition of terrestrial and aquatic *n*-alkanes to calculate a RH_{air} record from the Allerød-Younger Dryas-Early Holocene transition, which were so far only qualitatively interpreted (Rach et al., 2014).

Limitations of single isotope approaches

The hydrogen isotopic composition of terrestrial leaf wax-derived lipid biomarkers (such as long-chain *n*-alkanes and *n*-alkanoic acids) are used as source water hydrogen isotope composition recorders, and are therefore often interpreted as $\delta^2\text{H}_{\text{precipitation}}$ records (e.g. Jacob et al., 2007; Seki et al., 2011; Rach et al., 2014; Muschitiello et al., 2015). Leaf wax $\delta^2\text{H}$ extracted from lacustrine surface sediments (Sauer et al., 2001; Huang et al., 2004; Sachse et al., 2004; Mügler et al., 2008; Rao et al., 2014) and from surface soils (Hou et al., 2008; Rao et al., 2009), also display high correlation with $\delta^2\text{H}_{\text{precipitation}}$. As lipid biomarkers are biosynthesized in the leaves of the plants, this correlation is based on a strong leaf water to precipitation (~plant source water) relationship which occurs when leaf water enrichment is of minor relevance (Sachse et al., 2012). Long-term $\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$ variations show distinct correlation to local air temperature changes in temperate regions (e.g. Stumpp et al., 2014). In tropical regions the precipitation amount seems to play an important role (Dansgaard, 1964; Rozanski et al., 1993), at least on a seasonal time-scale (Rozanski et al., 1996). Araguás-Araguás et al. (2000) point out that effects associated with the moisture source (e.g. the transport history of the moisture or the temperature at the moisture source area) have to be taken into account when $\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$ variations are interpreted. Thus, leaf wax $\delta^2\text{H}$ records in high latitudes were interpreted in terms of a temperature and moisture source proxy (Rach et al., 2014), while in low latitudes

(e.g. in South-East Africa) the interpretation follows the notion of the precipitation amount versus $\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$ (Scheffuß et al., 2011). However, when leaf water enrichment caused by evapotranspiration occurs, it is reflected in the leaf wax $\delta^2\text{H}$ signatures (e.g. Kahmen et al., 2013a) which is the reason single leaf wax $\delta^2\text{H}$ -based climate proxies are often interpreted only quantitatively. For a decade, also compound-specific $\delta^{18}\text{O}$ analysis of neutral sugar biomarkers are used in climate studies (Zech and Glaser, 2009; Zech et al., 2013; Zech et al., 2014a). Accordingly $\delta^{18}\text{O}_{\text{sugar}}$ can be interpreted comparable to the leaf wax $\delta^2\text{H}$. If the sugars can be associated with hemicellulose structures of higher vascular plants, they likely reflect the source water signal (i.e. precipitation) modified by evapotranspirative enrichment of leaf water (Tuthorn et al., 2014; Zech et al., 2014b).

Potential of the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ approach

Leaf water enrichment is strongly driven by the RH_{air} in the surrounding of the leaves (as reviewed e.g. by Cernusak et al., 2016), which provides large potential to derive quantitative RH values from biomarker-isotopes such as leaf waxes and sugars. Indeed, a coupled approach using cellulose $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values was developed to derive RH values (Voelker et al., 2014). This concept was also applied to sub-fossil wood samples to derive RH changes throughout the Last Glacial Maximum-Early Holocene transition. A conceptual approach of coupling $\delta^2\text{H}_{n\text{-alkane}}$ with $\delta^{18}\text{O}_{\text{sugar}}$ results in order to reconstruct $\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$, as well as the RH_{air} , was recently validated via an Argentinian topsoil climate transect study (Tuthorn et al., 2015). This so-called paleohygrometer approach has the potential to disentangle the source water signal from the modifications caused by leaf water enrichment, overcoming the limitations of single biomarker-isotope approaches (for further details see section 3 and Figure 1). Zech et al. (2013) for the first time introduced this concept to derive RH_{air} and $\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$ records for the last 220 ka from a permafrost paleosol sequence in North-East Siberia. Using n -alkane and sugar biomarkers to gain respective $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results has advantages compared to using cellulose for compound specific $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurements (e.g. Mayr, 2002). Namely, the new approach overcomes challenges associated with (i) the interpretation of cellulose $\delta^2\text{H}$ results as derived from measurable nitro cellulose derivatives (e.g. Sternberg, 1988) and (ii) the complexity of the extraction, purification and $\delta^{18}\text{O}$ measurements (Mayr, 2002; Saurer and Siegwolf, 2004; Wissel et al., 2008). One essential requirement for a successful application of the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach is the biomarker source determination. When n -alkane and sugar biomarkers extracted from lake sediments originate mainly from aquatic sources no quantitative RH reconstructions can be derived (Hepp et al., 2015). Still, disentangling between the lake source water isotope signal ($\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$) and alternations caused by lake water enrichment, both incorporated into the biomarkers, was possible. Overall, the lake water enrichment has to be interpreted in terms of evaporation versus precipitation amount changes (Hepp et al., 2015).

Given the above outlined limitations of single isotope approaches and the potential of the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ approach, the following research objectives were addressed within this PhD thesis:

- Validating the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach using i) leaf sample material from a climate chamber experiment conducted with three different plant species (Mayr, 2002) and ii) topsoil samples along an European climate transect from Southern Sweden to Southern Germany (Schäfer et al., 2016).

- Application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach to a terrestrial climate archive, namely the loess-like paleosol sequence Maundi from the Southern slopes of Mt. Kilimanjaro. This archive offered the potential to establish a first ~ 100 ka RH record for East Africa.
- Source identification of terrestrial versus aquatic sugar biomarkers in a lacustrine study. This forms the basis for the interpretation of $\delta^{18}\text{O}_{\text{sugar}}$ records established from lake sediments.
- Application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach to two lake sedimentary archives from the West Eifel maar lake region (Lake Gemündener Maar) and the Southern Black Forest region (Lake Bergsee) in order to derive quantitative hydroclimate information from the Late Glacial-Early Holocene transition.

2 Compound-specific isotope analyses of biomarkers

The 'online' coupling of gas chromatographs via combustion or pyrolysis reactors to isotope ratio mass spectrometers (GC-C,Py-IRMS) developed around 30 years ago (Glaser, 2005; Amelung et al., 2009) augmented compound-specific isotope analyses of biomarkers. While $\delta^{13}\text{C}_{\text{sugar}}$ (e.g. Gross and Glaser, 2004) or $\delta^{13}\text{C}_{n\text{-alkane}}$ and $\delta^2\text{H}_{n\text{-alkane}}$ (e.g. Sessions et al., 1999; Zech and Glaser, 2008) biomarker analyses are nowadays well established in numerous scientific communities ranging from soil and plant science over climate research to forensics (Sachse et al., 2012; Diefendorf and Freimuth, 2016; Tipple et al., 2016; Jansen and Wiesenberger, 2017; Pedentchouk and Zhou, 2018), compound-specific $\delta^{18}\text{O}$ analyses of biomarkers have been realized and published by only three scientific working groups worldwide, hitherto.

2.1 Lipid biomarker and compound-specific $\delta^2\text{H}_{n\text{-alkane}}$ analyses

In order to obtain total lipid extracts from the leaf (manuscript 1), soil (manuscript 2), loess-like paleosol (manuscript 3) and lake sediment samples (manuscripts 5 and 6) three different extraction procedures were performed, i.e. accelerated solvent extraction (manuscript 3; e.g. Zech and Glaser, 2008), microwave extraction (manuscripts 1, 2 and 5; e.g. Veggi et al., 2013) and soxhlet extraction (manuscript 6; e.g. Bourbonniere et al., 1997). Solid phase chromatography via silica gel columns (aminopropyl-silica-gel (Supelco, 45 μm) filled pipettes) are used to separate the total lipid extract into a nonpolar fraction (incl. n -alkanes), a more polar fraction (incl. alcohols) and an acid fraction (incl. n -alkanoic acids). Therefore, the columns were flushed with organic solvents of increasing polarity, i.e. n -hexane, dichloromethane:methanol in a ratio of 1:1 and acetic acid:diethyl ether in a ratio of 1:19 (Hou et al., 2008; Schäfer et al., 2016) to extract the trapped fractions. If necessary (manuscripts 1, 2 and 5), the nonpolar fraction was cleaned over silver-nitrate coated silica gel columns and zeolith columns (Geokleen) in order to obtain a clean n -alkane fraction. Quantification of the n -alkanes was performed on GC to mass spectrometer couplings (manuscripts 1 and 5) and GCs equipped with a flame ionization detector (manuscripts 2, 3 and 6). Compound-specific n -alkane $\delta^2\text{H}$ analyses were performed using

a gas chromatography-isotope ratio mass spectrometry technique consisting of GC online connected via ^2H -pyrolysis reactor to an IRMS. The reactor temperature is thereby set to 1000 °C (GC5 pyrolysis, combustion interface equipped with a CR (ChromeHD) reactor; manuscripts 1 and 5), 1420 °C (in a GC-IRMS coupling of a TRACE GC Ultra and a Delta V Plus IRMS (Thermo Fisher Scientific, Bremen, Germany); manuscript 2), 1445 °C (in a GC-IRMS system equipped with a HP 6890 GC and a DeltaPLUSXL IRMS; manuscript 3) and 1425 °C (for an empty ceramic tube 2H-pyrolysis reactor in the GC IsoLink interface (Thermo Fisher Scientific, Bremen, Germany); manuscript 6). Co-analysing *n*-alkane standard mixtures with known isotope composition (supplied by A. Schimmelmann, University of Indiana) was used for calibration of the lipid $\delta^2\text{H}$ results and checking the precision of the GC-IRMS system. Latter was also ensured by measuring the H_3^+ factor routinely, which stayed constant during the measurement periods.

2.2 Sugar biomarker and compound-specific $\delta^{18}\text{O}_{\text{sugar}}$ analyses

Monosaccharide sugar biomarkers were extracted hydrolytically using 4M trifluoric acid for 4 h at 105 °C from all samples, according to standard procedures (Guggenberger et al., 1994; Amelung et al., 1996). Afterwards, the extracts were cleaned and purified over glass fibre filters followed by XAD-7 (removal of humic-like compounds) and DOWEX 50WX8 (removal of cations) columns. After freezing and freeze-drying the samples, methylboronic acid derivatization was conducted (Knapp, 1979) in order to make the monosaccharides arabinose, fucose, xylose and rhamnose GC-amenable (Gross and Glaser, 2004), allowing for the compound-specific $\delta^{18}\text{O}$ analyses (Zech and Glaser, 2009). Compound-specific $\delta^{18}\text{O}_{\text{sugar}}$ analyses were performed on a GC-IRMS system that was coupled online via an ^{18}O -pyrolysis reactor, which was set to 1280 °C (Zech and Glaser, 2009). The co-analysis of external sugar standards containing arabinose, fucose, xylose and rhamnose in different concentrations and of known isotope compositions ensured the 'Principle of Identical Treatment' standard for stable isotope analysis (according to Werner and Brand, 2001), and allowed furthermore the correction for possibly occurring amount effects (according to Zech and Glaser, 2009).

The compound-specific isotope results are expressed in the common delta notation ($\delta = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}$, where $R = ^{18}\text{O}/^{16}\text{O}$ or $^2\text{H}/^1\text{H}$), relative to the Vienna Standard Mean Ocean Water standard (VSMOW; Coplen, 2011).

3 Principle of the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach

3.1 Biomarker-based leaf water reconstructions

The fundamental assumption of the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach is that biomarker isotope signatures reflect primarily the leaf water isotope composition (Figure 1). Hence, when applying biosynthetic fractionation factors leaf water can be reconstructed. Fractionation factors can be derived from the literature. Most likely, appropriate factors are -160‰ for ^2H of *n*-alkanes ($\varepsilon_{\text{bio}}^2$; Sessions et al., 1999; Sachse et al.,

2012) and +27‰ for ^{18}O in sugars ($\epsilon_{\text{bio}}^{18}$ Sternberg et al., 1986; Yakir and DeNiro, 1990; Schmidt et al., 2001; Cernusak et al., 2003):

$$\delta\text{H}_{\text{leaf-water}}^2 = (\delta^2\text{H}_{n\text{-alkane}} - \epsilon_{\text{bio}}^2) / (1 + \epsilon_{\text{bio}}^2/1000) \quad (1)$$

$$\delta^{18}\text{O}_{\text{leaf-water}} = (\delta^{18}\text{O}_{\text{sugar}} - \epsilon_{\text{bio}}^{18}) / (1 + \epsilon_{\text{bio}}^{18}/1000) \quad (2)$$

The n -alkane and sugar biomarkers can be related to the epicuticular leaf wax layers (Eglinton and Hamilton, 1967) and to the (leaf) hemicellulose structures of higher vascular plants (e.g. Jia et al., 2008; Zech et al., 2012; Hepp et al., 2016, and references therein), respectively. A large overview of plant cell wall structure and chemical composition is e.g. given in Caffall and Mohnen (2009). The biomarkers thus incorporate the isotope signal of the (leaf) water in which they are biosynthesized. Accordingly the leaf wax n -alkanes and leaf (hemi-)celluloses primarily reflect bulk leaf water during the photosynthetic active period (e.g. Barbour and Farquhar, 2000; Roden et al., 2000; Schmidt et al., 2003; Cernusak et al., 2005; Sachse et al., 2012; Kahmen et al., 2013a).

3.1.1 Excursus: Uncertainties of the $\delta^2\text{H}_{n\text{-alkane}}$ to $\delta^2\text{H}_{\text{leaf-water}}$ relationship

The main uncertainty for n -alkane-based leaf water reconstructions is most likely associated with the rather variable $\delta^2\text{H}_{n\text{-alkane}}$ to $\delta^2\text{H}_{\text{leaf-water}}$ relationship, which results in a large range of ϵ_{bio}^2 (also called $\epsilon_{n\text{-alkane/leaf-water}}$), as presented in the literature (e.g. Feakins and Sessions, 2010; Tipple et al., 2015; Feakins et al., 2016; Freimuth et al., 2017). Based on the therein published $\epsilon_{n\text{-alkane/leaf-water}}$ data of the n -alkanes C_{29} and C_{31} , a large variation of approximately 174‰ (ranging from -66 to -240‰) with a median close to -155‰ can be derived. Furthermore, the timing of leaf wax synthesis could contribute to the wide range of observed $\epsilon_{n\text{-alkane/leaf-water}}$ values. If leaf wax synthesis occurs mainly during leaf flush (Tipple et al., 2013; Gamarra and Kahmen, 2017), the n -alkanes incorporate the source (leaf) water signal during that period (Sachse et al., 2010) and can therefore not be well correlated to the leaf water signals during the whole growing season. For long-chain leaf lipids (from C_{27} to C_{31}), a complete recycling could occur after 71 to 128 days as derived from a labeling experiment of the grass species *Phleum pratense* conducted by Gao et al. (2012). In addition, the influence of storage carbohydrates on the isotope signature of the n -alkanes biosynthesized during leaf flush might be increased (Newberry et al., 2015). However, there is also evidence that n -alkanes reflect more or less continuously the climate conditions during the whole vegetation period (e.g. Newberry et al., 2015; Pedentchouk and Zhou, 2018, and references therein). The n -alkanes of grass species tend to reflect only partially the leaf water signal (Kahmen et al., 2013a; Gamarra et al., 2016). They are considered to be mainly influenced by the source water of the plants (McInerney et al., 2011) due to their leaf growth at the intercalary meristem located at the base of the leaves. Finally, species-specific differences in ϵ_{bio}^2 have to be taken into account (see reviews from Schmidt et al. (2003), Sachse et al. (2012), and Pedentchouk and Zhou (2018), and reference therein), exemplary highlighted by stomatal density effects (Lee et al., 2016) and the carbon as well as the energy metabolism of plants (Cormier et al., 2018; Tipple and Ehleringer, 2018) on ϵ_{bio}^2 .

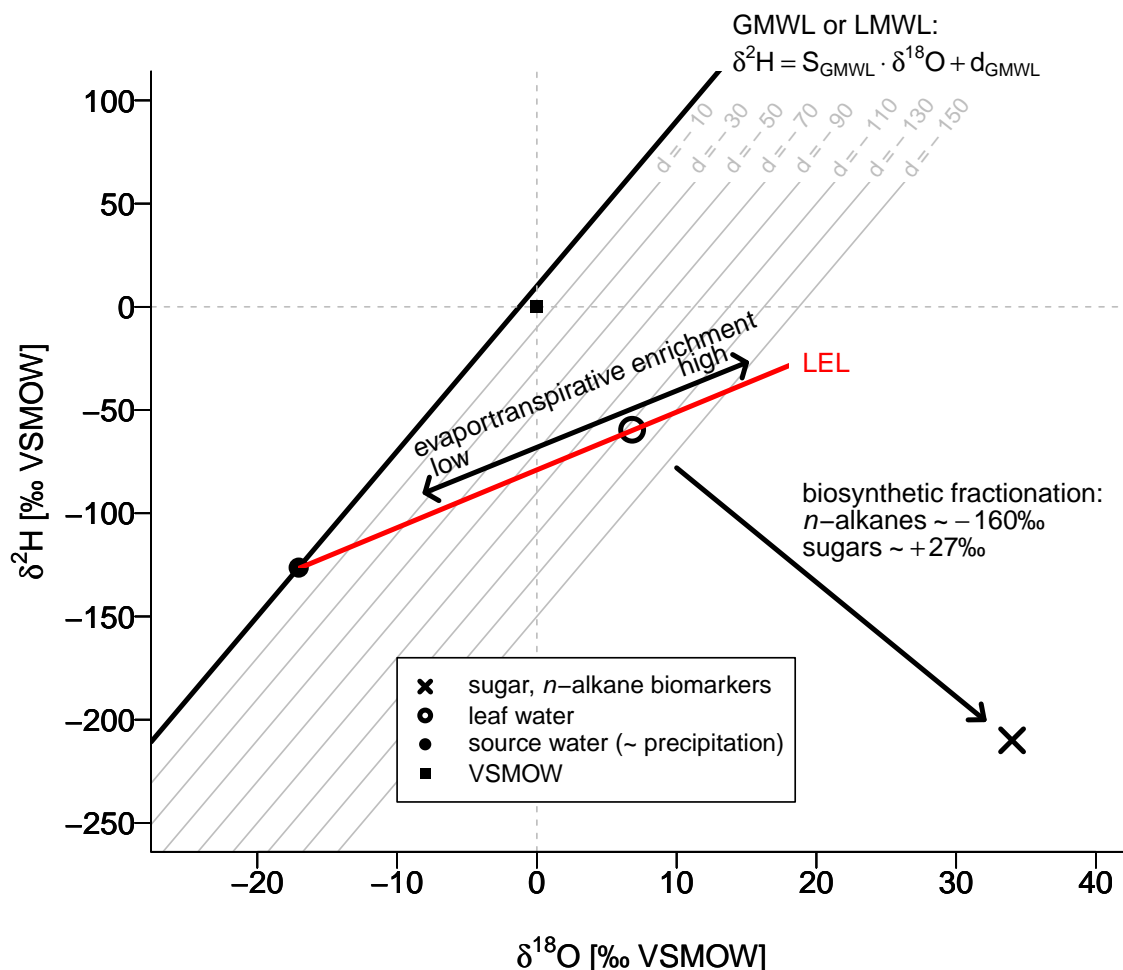


Figure 1: $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ diagram illustrating (i) $\delta^2\text{H}_{n\text{-alkane}}$ versus $\delta^{18}\text{O}_{\text{sugar}}$ (marked with a cross), (ii) the reconstructed leaf water isotope composition by applying biosynthetic fractionation factors (Sessions et al., 1999; Sachse et al., 2012; Schmidt et al., 2001; Cernusak et al., 2003); marked with an open circle), (iii) the possibility to reconstruct source water isotope composition, which could serve as a proxy for precipitation isotope composition, using the intersect between the local evaporation line (LEL) and the global or local meteoric water line (GMWL or LMWL; marked with a filled circle), and finally (iv) the effect of low and high leaf water evapotranspirative enrichment along the LEL on the deuterium-excess (d) of leaf water (which is the parallel distance to the GMWL or LMWL, marked with d letters), possibly serving as quantitative RH proxy (Zech et al., 2013; Tuthorn et al., 2015).

3.1.2 Excursus: Uncertainties of the $\delta^{18}\text{O}_{\text{sugar}}$ to $\delta^{18}\text{O}_{\text{leaf-water}}$ relationship

Uncertainties for reconstructing $\delta^{18}\text{O}_{\text{leaf-water}}$ based on (leaf) hemicellulose sugar $\delta^{18}\text{O}$ are mostly based on cellulose studies. Overall, the influence of (unenriched) source water, which is commonly less enriched than the leaf water, cannot be ruled out. This holds true for dicotyledon plants (e.g. reviews from Barbour, 2007; Sternberg et al., 2006), monocotyledon (grass) species (Helliker and Ehleringer, 2002; Liu et al., 2016; Liu et al., 2017; Lehmann et al., 2017), and surely for stem cellulose (e.g. Roden et al., 2000), but also for leaf cellulose (Wang et al., 1998; Barbour and Farquhar, 2000; Cernusak et al., 2005; Song et al., 2014; Cheesman and Cernusak, 2017; Munksgaard et al., 2017). However, large ranges of the so called damping factor are reported for empirical data (Wang et al., 1998) but also with regard to the theory (Song et al., 2014). The theoretical approach for $\delta^{18}\text{O}$ in (hemi-)cellulose is based on the premise that sucrose exported from photosynthesizing leaves is +27‰ more positive compared to leaf water (Cernusak et al., 2003), which is interpreted as sucrose being in full isotopic equilibrium with the synthesis water. Latter is drawn from the comparison to the equilibrium fractionation effect of the reversible hydration reaction of acetone, which contains only one exchangeable oxygen, with water, resulting in an enrichment of +28, +28 and +26‰ at 15, 25 and 35°C, respectively (Sternberg and DeNiro, 1983).

Also the cellulose biosynthesis is associated with an enrichment of around +27‰ compared to the synthesis water as shown in growth experiments (Sternberg et al., 1986; Yakir and DeNiro, 1990), which is again generally explained via the isotope exchange between the carbonyl oxygen and water (Schmidt et al., 2001). This means that the isotope signal of the leaf water incorporated by the transport sugar sucrose can potentially be dampened by oxygen exchange with local synthesis water during autotrophic, in terms of synthesized from photosynthesis products (*sensu* Terwilliger et al., 2002), (hemi-)cellulose biosynthesis in any sink tissue. This can be described by the equation of Barbour and Farquhar (2000): $\delta^{18}\text{O}_{(\text{hemi-})\text{cellulose}} = \delta^{18}\text{O}_{\text{source-water}} + (\delta^{18}\text{O}_{\text{leaf-water}} - \delta^{18}\text{O}_{\text{source-water}}) \cdot (1 - p_{\text{ex}} \cdot p_x) + \epsilon_{\text{bio}}^{18}$. Herein, p_x is the proportion of unenriched source water contribution to the local synthesis water and p_{ex} is the proportion of exchangeable oxygen during cellulose synthesis, in multiplication called damping factor.

The exchange is caused by hydration reactions that affect one oxygen when sucrose is cleaved into glucose phosphate, via the reversible conversion to fructose 6-phosphate and fructose 1,6-biphosphate (Waterhouse et al., 2013). A portion of fructose 1,6-biphosphate undergoes a futile cycling through triose phosphates, which allows further three oxygen positions to exchange (Barbour and Farquhar, 2000; Barbour, 2007; Sternberg, 2009; Waterhouse et al., 2013). However the transfer of the cellulose directly to hemicellulose $\delta^{18}\text{O}$ has to be questioned. Pentoses, like the hemicellulose-derived arabinose and xylose, are biosynthesized via decarboxylation of the carbon at position six (C6) from glucose (Altmatt and Neish, 1956; Harper and Bar-Peled, 2002; Burget et al., 2003). Waterhouse et al. (2013) suggested that the oxygens at this glucose C6 position are most strongly affected by the exchange with local water medium (as indicated by 80% exchange during heterotrophic cellulose synthesis).

Thus, most likely at least $\delta^{18}\text{O}$ of hemicellulose-derived pentoses are less effected by potential unenriched source water exchange processes. Still, for stem hemicelluloses from dicotyledonous plants, which grew under controlled climate conditions, a damping factor of

50 to 81% was observed (as highlighted by Sternberg, 2014, based on the data published by Zech et al., 2014b). From the presented theory it is also evident, that biosynthetic fractionation ($\varepsilon_{\text{bio}}^{18}$, and also called $\varepsilon_{\text{sugar/leaf-water}}^{18}$) effects the $\delta^{18}\text{O}_{\text{sugar}}$ signature. Indeed, the temperature dependency of $\varepsilon_{\text{bio}}^{18}$ is still under debate (Sternberg and Ellsworth, 2011; Sternberg, 2014 versus Zech et al., 2014c). So far there is evidence that the $\delta^{18}\text{O}$ signature of storage substances like starch, which indeed contribute to leaf cellulose synthesis (e.g. Terwilliger et al., 2001, sensu Terwilliger et al., 2002), can also be described via an +27‰ enriched compared to the synthesis water (as e.g. summarized and suggest by Sternberg (2009). However, the question how strong the $\delta^{18}\text{O}$ imprint of such storage substances is related to $\delta^{18}\text{O}_{\text{leaf-water}}$ is even more important, which cannot be answered here (see e.g. Sternberg et al., 2006; Lehmann et al., 2017, for more details). Sucrose synthesis gradients within a leaf, as well as leaf water inhomogeneity, could lead to weakening the $\delta^{18}\text{O}_{\text{leaf-water}}$ to $\delta^{18}\text{O}_{\text{sugar}}$ relationship, highlighted by a recent study by Lehmann et al. (2017) showing that the bulk leaf water is not always a good substitute of cellulose synthesis water in leaves. Finally, such leaf water inhomogeneities tend to increase under decreasing RH conditions, and vice versa (Santrucek et al., 2007), affecting not only the $\delta^{18}\text{O}_{\text{leaf-water}}$ to $\delta^{18}\text{O}_{\text{sugar}}$ correlation but also the $\delta^2\text{H}_{\text{leaf-water}}$ to $\delta^2\text{H}_{\text{n-alkane}}$ relation.

3.2 Leaf water enrichment theory

The second basic assumption of the paleohygrometer approach concerns leaf water enrichment. Leaf water is commonly enriched compared to the source water utilized by the plants during day time (e.g. the review of Cernusak et al., 2016). This is caused by the evaporation process while the plants transpire water through the stomata (Figure 1). As the leaf water reservoir close to the stomata (at the site where the evaporation takes place) is rather small, it can be assumed that the steady-state conditions occur rather rapidly (Allison et al., 1985; Walker and Brunel, 1990; Bariac et al., 1994; Gat et al., 2007). With the isotope composition of the transpired water being equal to the source water of the plants, the leaf water enrichment can be described via a Craig-Gordon model, given here in δ terms (Equation 3; Flanagan et al., 1991; Roden and Ehleringer, 1999; Barbour et al., 2004):

$$\delta_e \approx \delta_s + \varepsilon^* + \varepsilon_k + (\delta_a - \delta_s - \varepsilon_k) \cdot e_a/e_i. \quad (3)$$

Here, δ_e , δ_s and δ_a are the isotope compositions of evaporative site leaf water, source water and atmospheric water vapor, respectively. The equilibrium enrichment (expressed as $(1-1/\alpha_{\text{LV}}) \cdot 10^{-3}$, where α_{LV} is the equilibrium fractionation factor between liquid water and water vapour) is included as ε^* in ‰. The kinetic fractionation parameters, describing the water vapor diffusion through the stomata and the boundary air layer is expressed as ε_k in ‰. Finally, e_a/e_i is the ratio of atmospheric vapor pressure to intracellular vapor pressure, hence a leaf RH realisation. As the ε_k values are unknown for paleo applications, due to their dependency on stomatal and boundary layer resistances to the water (vapor) flux (Farquhar et al., 1989), it seems to be appropriate to use more general defined kinetic enrichment parameters (C_k) instead (Craig and Gordon, 1965; Gat and Bowser, 1991). Assuming that leaf temperature is equal to air temperature, the e_a/e_i ratio can be replaced by the air RH. If finally an isotope equilibrium between the source water of the plants and the local atmospheric water vapour is hypothesised, then the term $\delta_a - \delta_s$ can be approximated with $-\varepsilon^*$, thus Equation 3 will be simplified to:

$$\delta_e \approx \delta_s + (\varepsilon^* + C_k) \cdot (1 - \text{RH}) \quad (4)$$

3.3 Reconstruction of plant source water $\delta^2\text{H}$, $\delta^{18}\text{O}$ and RH_{air}

With the above-presented leaf water enrichment model at hand, both the isotopic composition of plant source water and RH can be reconstructed. Plant source water can be directly linked to soil water and shallow groundwater, which in turn reflect mean annual precipitation (e.g. Herrmann et al., 1987). The isotope composition of global precipitation plots typically along the global meteoric water line (GMWL, with the equation $\delta^2\text{H}_{\text{precipitation}} = 8 \cdot \delta^{18}\text{O}_{\text{precipitation}} + 10$; Dansgaard, 1964) and the isotope composition of local precipitation plots along a local meteoric water line (LMWL, with various equations depending on the locality). These observations can be used for inferring information about the source water of the plants (Figure 1). Plant source water isotope composition can thereby be calculated as the intersect between the local evaporation line, on which the leaf water plots, and the GMWL (Zech et al., 2013) or LMWL. With regard to Equation 4, the slope of the LEL (S_{LEL}) can be derived from Equation 5

$$S_{\text{LEL}} = (\delta_e^2 - \delta_s^2) / (\delta_e^{18} - \delta_s^{18}) \approx (\epsilon_2^* + C_k^2) / (\epsilon_{18}^* + C_k^{18}). \quad (5)$$

The equilibrium fractionation parameters (ϵ_2^* and ϵ_{18}^*) can be calculated according to temperature dependent empirical equations from Horita and Wesolowski (1994). The kinetic fractionation factors (C_k^2 and C_k^{18}) can be derived from Merlivat (1978), who reported maximum values of the molecular water diffusion through a stagnant boundary layer, which seems to be appropriate for leaves. It should be noted that ϵ_k values for broad-leaf trees and shrubs are well in range with the used C_k values (as derived from the supplementary data of Cernusak et al. (2016)). Calculated LEL slopes (Equation 5), only depending on the temperature via the equilibrium fractionation parameters, are well in range with slopes observed in the field and in the laboratory experiments (Zech et al., 2013; Tuthorn et al., 2015 versus Allison et al., 1985; Walker and Brunel, 1990; Bariac et al., 1994; Gat et al., 2007; Tiple et al., 2013). Using the deuterium-excess (d) definition of either the GMWL ($d = \delta^2\text{H} - 8 \cdot \delta^{18}\text{O}$; Dansgaard, 1964) or the LMWL ($d = \delta^2\text{H} - \text{slope of LMWL} \cdot \delta^{18}\text{O}$), the leaf water enrichment model can be described for hydrogen as well as oxygen in a single equation, which can be rearranged in order to calculate RH values (Zech et al., 2013):

$$\text{RH} \approx 1 - (d_e - d_s) / (\epsilon_2^* - S_{\text{GMWL,LMWL}} \cdot \epsilon_{18}^* + C_k^2 - S_{\text{GMWL,LMWL}} \cdot C_k^{18}). \quad (6)$$

Here, d_e and d_s are the deuterium-excess of evaporative site leaf water and source water, respectively, and slopes of the GMWL or LMWL are given as $S_{\text{GMWL,LMWL}}$. When d_s values are achievable from the GMWL or LMWL, then a powerful tool is given for deriving past RH changes via d_e (Zech et al., 2013; Tuthorn et al., 2015), under the assumption that d_e can be derived from the biomarker-based $\delta^2\text{H}_{\text{leaf-water}}$, $\delta^{18}\text{O}_{\text{leaf-water}}$ reconstructions (Equation 1 and Equation 2). Equation 6 requires strictly seen d_e values as input, while the biomarker-based leaf water results rather in a deuterium-excess of (bulk) leaf water (d_l). The d_l values are most likely less enriched than the deuterium-excess at the evaporative site (d_e). It should be noted that differences between d_e and d_l are far below typical analytic errors associated with compound-specific $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ analysis, as derived from d_e and d_l assessment using the complete data sets from Australia (Kahmen et al., 2013b) and Hawaii (Kahmen et al., 2011a), as presented in the supplementary data of Cernusak et al. (2016). The d values were here calculated via a local deuterium-excess formulation using the presented $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of xylem water. Thus a correction

of biomarker-derived d_l values in order to achieve d_e as input for Equation 6 seems to be unnecessary. The here presented approach allows reconstructing RH (particularly of day-time and vegetation time, see Tuthorn et al. (2015) and $\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$ (particularly of weighted mean annual precipitation).

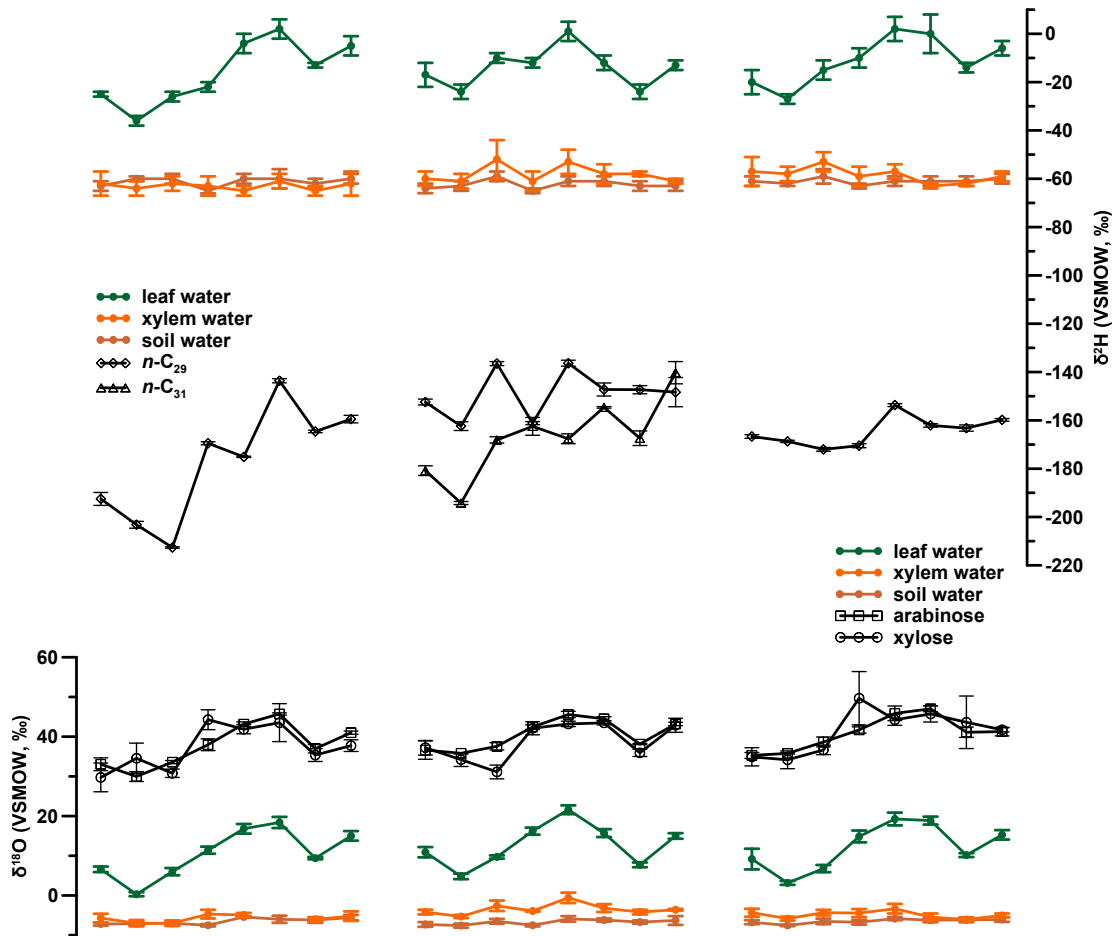
4 Results & Discussion

4.1 Climate chamber validation study 1 (manuscript 1)

The overall aim of this study was to validate the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach by using leaf material from plants grown under controlled climate conditions. A climate chamber experiment, conducted by co-author Christoph Mayr at the Helmholtz Zentrum München during winter 2000/2001 (Mayr, 2002), was used to investigate leaf samples for their $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ values. The three different plant species used in the experiment (*Eucalyptus globulus*, *Vicia faba* var. *minor* and *Brassica oleracea* var. *medullosa*) were grown under seven air temperature (T_{air}) and RH_{air} conditions (14, 18, 24 and 30 °C; 21, 24, 32, 48, 49, 50 and 68%) for 56 days. After this period, the plants were harvested and analyzed for $\delta^2\text{H}_{\text{leaf-water}}$, $\delta^{18}\text{O}_{\text{leaf-water}}$. For more details about the experimental set-up and plant related results see Mayr (2002). The analyzed alkanes $n\text{-C}_{29}$ and $n\text{-C}_{31}$ can be associated with the epicuticular leaf wax layers of the plants (Eglinton and Hamilton, 1967), while the extracted monosaccharides arabinose and xylose originate from the hemicellulose structure of the plant leaf cells (Caffall and Mohnen, 2009). The $n\text{-C}_{29}$ and $n\text{-C}_{31}$ $\delta^2\text{H}$ results were combined as weighted mean to $\delta^2\text{H}_{n\text{-alkane}}$ values and the arabinose and xylose $\delta^{18}\text{O}$ values were used to calculate weighted mean $\delta^{18}\text{O}_{\text{sugar}}$ results (Figure 2).

Both biomarker isotope values are highly significantly correlated with the respective leaf water isotope values ($\delta^2\text{H}_{n\text{-alkane}}$ versus $\delta^2\text{H}_{\text{leaf-water}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ versus $\delta^{18}\text{O}_{\text{leaf-water}}$: $r^2 = 0.45$ and 0.85 , $p < 0.001$, $n = 24$). The mean fractionation factors derived from the difference between the biomarkers and the leaf water were -156‰ (within a range from -192 to -133‰) for $\epsilon_{n\text{-alkane/leaf-water}}$ and $+27.3\text{‰}$ for $\epsilon_{\text{sugar/leaf-water}}$ (within a range from 23.0 to 32.3‰), which are well in agreement with the literature (Sessions et al., 1999; Kahmen et al., 2011b; Sachse et al., 2012; Sternberg et al., 1986; Yakir and DeNiro, 1990; Schmidt et al., 2001; Cernusak et al., 2003).

In order to evaluate if the principle assumption of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach is valid (as outlined in detail in section 3), the measured $\delta^2\text{H}_{\text{leaf-water}}$ and $\delta^{18}\text{O}_{\text{leaf-water}}$ results were used as input variables for calculating RH_{air} via Equation 6 (Figure 3A). The d_e values were derived from the equation $d_e = \delta\text{H}_{\text{leaf-water}}^2 - 8 \cdot \delta^{18}\text{O}_{\text{leaf-water}}$ (according to the equation of Dansgaard (1964) and the GMWL was used as baseline, revealing a d of 10 (used for d_s) and a slope of 8 (Craig, 1961). Modeled RH_{air} values fit very well with the measured RH_{air} values along the 1:1 line (Figure 3A; $R^2 = 0.84$, RSME = 6.04%). When biomarker-based $\delta^2\text{H}_{\text{leaf-water}}$, $\delta^{18}\text{O}_{\text{leaf-water}}$ values are used (calculated via Equation 1 and Equation 2) for RH_{air} modeling, the RH_{air} predictions are getting worse, but are still significant (Figure 3B, modified from Hepp et al., 2019c). The weaker R^2 when biomarker-based $\delta^2\text{H}_{\text{leaf-water}}$, $\delta^{18}\text{O}_{\text{leaf-water}}$ values are used in comparison to measured values points to one main uncertainty of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer

A) water and biomarker $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values

B) climate chamber conditions

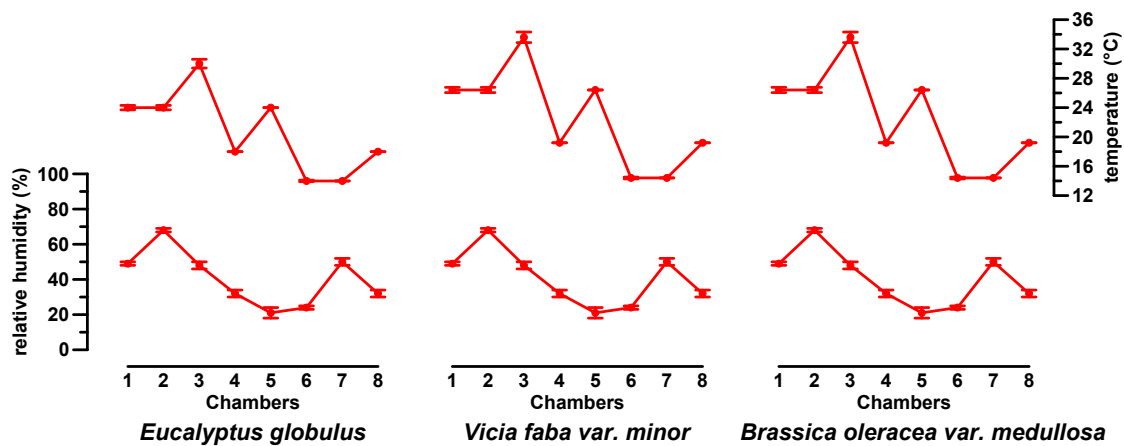


Figure 2: Plant water and climate chamber conditions of the controlled experiment (modified from Hepp et al., 2019c). A) Leaf, xylem and soil water isotope composition (all from Mayr, 2002), along with respective $\delta^2\text{H}_{n\text{-alkane}}$ ($n\text{-C}_{29}$ and $n\text{-C}_{31}$) and $\delta^{18}\text{O}_{\text{sugar}}$ (arabinose and xylose) values. B) Air temperature and relative humidity conditions of the climate chambers (Mayr, 2002).

approach, which is related to the limitations associated with the biomarker-based leaf water reconstruction (see paragraph above). This is understandable when considering the large ranges of the observed $\varepsilon_{n\text{-alkane/leaf-water}}$ and $\varepsilon_{\text{sugar/leaf-water}}$ values as well as the fairly well $\delta^2\text{H}_{n\text{-alkane}}$ to $\delta^2\text{H}_{\text{leaf-water}}$ relationship. Still, the high potential of the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach is highlighted by robust RH reconstructions, considering an R^2 of 0.54 for the biomarker-based versus measured RH_{air} relationship and an RSME of 10.14% (Figure 3B).

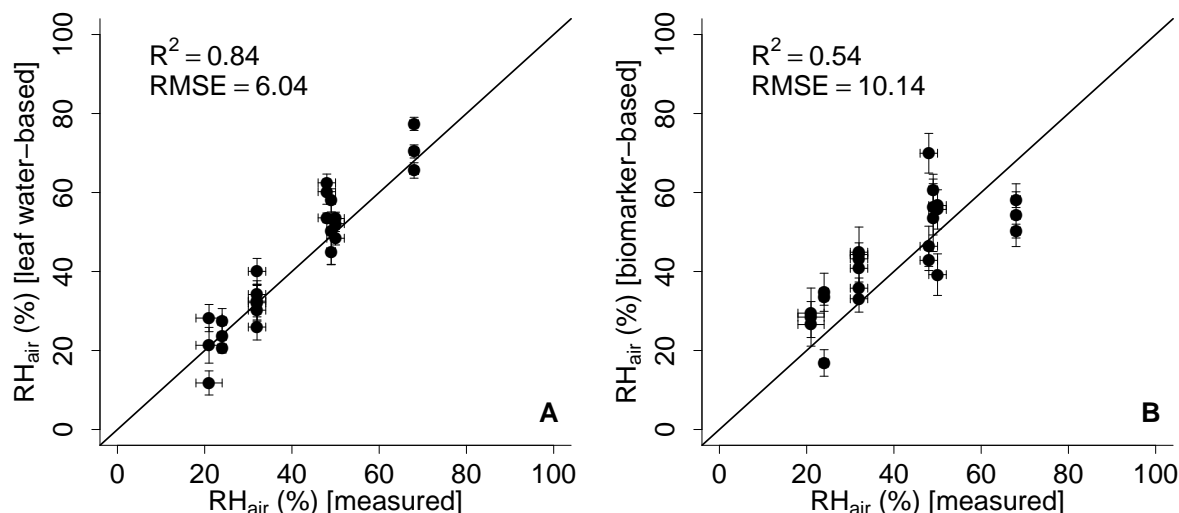


Figure 3: Scatterplots of leaf water- (A) and biomarker-based (B) relative air humidity values (RH_{air}) versus measured RH_{air} values (modified from Hepp et al., 2019c). Black line = 1:1 line; R^2 = coefficient of correlation along the 1:1 line; RMSE = root mean square error in % RH_{air} .

4.2 European climate transect validation study 2 (manuscript 2)

The European topsoil climate transect, established by Imke Kathrin Schäfer and co-authors, allows evaluating the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ approach under field conditions. For establishing the transect reaching from Southern Sweden to South Germany, topsoil samples (0-5 cm of the Ah horizons) at 16 locations were taken in November 2012. Furthermore, three different vegetation types, i.e. coniferous forest, deciduous forest and grassland were differentiated, which leads in summary to 29 sampling points. Climate variables were derived from the close-by climate station data (climate data were retrieved from the respective German, Danish and Swedish weather observation institutions (DWD, DMI and SMHI); Frich et al., 1997; Laursen et al., 1999; Cappelen, 2002; DWD Climate Data Center, 2018b; DWD Climate Data Center, 2018a; Swedish Meteorological and Hydrological Institute, 2018). From this database, climate variability along the transect regarding long-term mean annual temperatures (T_{MA}) and RH (RH_{MA}), long-term means for the vegetation period (April to October; T_{MV} , RH_{MV}) and finally long-term mean for the day-time (from 7 a.m. to 7 p.m.) and vegetation period (T_{MDV} , RH_{MDV}), were obtained. In addition, long-term mean annual precipitation amount (P_{MA}) was calculated. For two Danish sites long-term means of T_{MDV} were not available (the T_{MV} were used instead). Along the transect, T_{MA} range from 5.3 to 10.6°C and mean annual precipitation (P_{MA}) ranges from 554 to

1769 mm, which is quite comparable to a published Argentinian transect which was used for validating the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach (Tuthorn et al., 2015). However, the (weighted) mean annual isotopic composition of precipitation shows smaller variations along the European transect compared to the Argentinian transect. For the Swedish and Danish sites of the European transect, $\delta^2\text{H}_{\text{precipitation}}$ data was gathered from the The Online Isotopes in Precipitation Calculator (called $\delta^2\text{H}_{\text{OIPC}}$, $\delta^{18}\text{O}_{\text{OIPC}}$; Bowen and Revenaugh, 2003; IAEA/WMO, 2015; Bowen, 2018). For the German sites, a regional precipitation $\delta^2\text{H}$ and $\delta^{18}\text{O}$ regionalisation was realized by using long-term data available from 34 German GNIP stations, 4 Austrian ANIP stations (Reutte, Scharnitz, Salzburg, Kufstein) and from Groningen GNIP station, (Stumpp et al., 2014; Geldern et al., 2014; IAEA/WMO, 2018; Umweltbundesamt GmbH, 2018), referred to as $\delta^2\text{H}_{\text{GIPR}}$, $\delta^{18}\text{O}_{\text{GIPR}}$, for more details see manuscript 2. $\delta^2\text{H}_{\text{GIPR,OIPC}}$ and $\delta^{18}\text{O}_{\text{GIPR,OIPC}}$ varies between -52 and -79‰ (= 27‰ range) and between -7.4 and -10.9‰ (= 3.5‰ range), respectively. Along the Argentinian transect, the $\delta^2\text{H}_{\text{OIPC}}$ and $\delta^{18}\text{O}_{\text{OIPC}}$ ranges from -29 to -87‰ (= 58‰ range) and from -5.0 to -11.7‰ (= 6.7‰ range), which is approximately double as large (Tuthorn et al., 2014; Tuthorn et al., 2015). Similar findings are reported for the mean annual RH gradient, which is 25% regarding RH_{MA} along the Argentinian sites (Tuthorn et al., 2015), whereas along the European transect study only a 12% variation can be observed. Therefore, the European topsoil transect can also be seen as a sensitivity test for the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach. In summary, 25 samples could be used for coupling of $\delta^2\text{H}_{n\text{-alkane}}$ with $\delta^{18}\text{O}_{\text{sugar}}$ results, which yielded in biomarker-based $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ (~precipitation) and RH_{MDV} values. For $\delta^2\text{H}_{n\text{-alkane}}$ the mean of $\delta^2\text{H}$ of $n\text{-C}_{27}$, $n\text{-C}_{29}$ and $n\text{-C}_{31}$ was used, while for $\delta^{18}\text{O}_{\text{sugar}}$, arabinose and xylose $\delta^{18}\text{O}$ results were combined as weighted means.

The apparent isotope fractionation, calculated as $\varepsilon_{n\text{-alkane/precipitation}} = (\delta^2\text{H}_{n\text{-alkane}} - \delta^2\text{H}_{\text{GIPR,OIPC}})/(1 + \delta^2\text{H}_{\text{GIPR,OIPC}}/1000)$ and $\varepsilon_{\text{sugar/precipitation}} = (\delta^{18}\text{O}_{\text{sugar}} - \delta^{18}\text{O}_{\text{GIPR,OIPC}})/(1 + \delta^{18}\text{O}_{\text{GIPR,OIPC}}/1000)$, is lower for sugars and more negative for $n\text{-alkanes}$ from grassland compared to the forest sites. This means that $\varepsilon_{n\text{-alkane/precipitation}}$ and $\varepsilon_{\text{sugar/precipitation}}$ from the grassland sites is closer to the expected $\varepsilon_{\text{bio}}^2$ and $\varepsilon_{\text{bio}}^{18}$ values of -160‰ (Sessions et al., 1999; Sachse et al., 2012) and +27‰ (Sternberg et al., 1986; Yakir and DeNiro, 1990; Schmidt et al., 2001; Cernusak et al., 2003). This finding is well in agreement with recent studies showing that $n\text{-alkanes}$ and cellulose extracted from grass leaves are less sensitive leaf water recorders (McInerney et al., 2011; Kahmen et al., 2013a; Gamarra et al., 2016; Helliker and Ehleringer, 2002). Hence, grass biomarkers reflect the more negative source water signal (~precipitation) rather than the leaf water, which is influenced by evapotranspirative enrichment. Most likely the basal growth form of grass species via an intercalary meristem can explain this effect. Indeed, water sampled from the leaf growth and differentiation zone is close to the source water of grasses (Liu et al., 2017).

The biomarker-based $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ values plot reasonable well close to the 1:1 lines with $\delta^2\text{H}_{\text{GIPR,OIPC}}$ and $\delta^{18}\text{O}_{\text{GIPR,OIPC}}$ but show a much larger range (93 and 12‰ for $\delta^2\text{H}_{\text{source-water}}$ and $\delta^{18}\text{O}_{\text{source-water}}$, respectively; Figure 4A and B). The same holds true for the comparison of biomarker-based with climate station RH_{MVD} . While RH_{MVD} derived from the climate stations show a variation of 17%, the biomarker-based RH_{MVD} show a total range of 40% (Figure 4C). The larger range in the reconstructions could be caused by uncertainties associated with the $\delta^2\text{H}_{\text{leaf-water}}$ reconstruction based on $n\text{-alkane}$ $\delta^2\text{H}$ and a constant $\varepsilon_{\text{bio}}^2$ factor (see also section 3.1.1). Also the usage of the same LEL slope for the coniferous sites as for deciduous tree and grass sites could lead to the observed large

ranges in the reconstructions. Moreover, systematic offsets between the reconstructed $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and RH_{MDV} values compared to the GIPR, OIPC precipitation values and the climate station-derived RH_{MDV} are obvious (median $\Delta \delta^2\text{H} \approx -21\text{‰}$, $\Delta \delta^{18}\text{O} \approx -2.9\text{‰}$ and $\Delta \text{RH}_{\text{MDV}} \approx -17.1\%$). With regard to reconstructed $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ this could be caused by the usage of a too steep LEL slope for the coniferous sites. Furthermore, the consideration of the loss of evaporative leaf water enrichment would diminish the negative offset of the grass sites. The study shows therefore the limitations of reconstructing medium variations in precipitation $\delta^2\text{H}$, $\delta^{18}\text{O}$ and RH_{MDV} by using the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach.

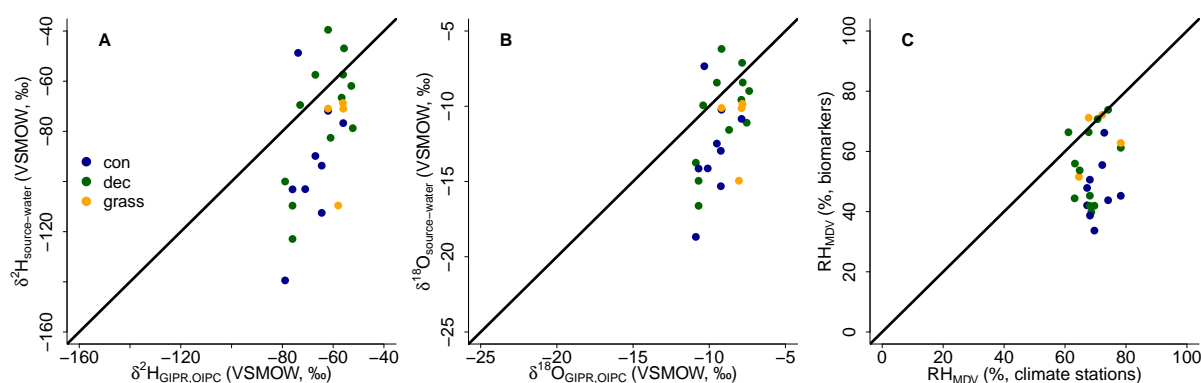


Figure 4: Scatterplots comprising reconstructed source water $\delta^2\text{H}$ versus $\delta^2\text{H}_{\text{GIPR,OIPC}}$ (A), reconstructed source water $\delta^{18}\text{O}$ versus $\delta^{18}\text{O}_{\text{GIPR,OIPC}}$ (B) and reconstructed RH_{MDV} based on the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach versus climate station RH_{MDV} values (C). Abbreviations: con = coniferous forest sites; dec = deciduous forest sites; grass = grassland sites, modified from Hepp et al. (2019b).

4.3 Application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach to the terrestrial sedimentary archive of Maundi (manuscript 3)

A first application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach within this PhD project was realized on a loess-like paleosol sequence from the Southern slopes of Mt. Kilimanjaro called Maundi (2780 m a.s.l.; 3°10'27.5"S, 37°31'05.8"E). The age-depth model suggests that the Maundi record covers approximately the last 100 ka. The sequence was analyzed for $\delta^{18}\text{H}_{\text{sugars}}$ and $\delta^2\text{H}_{\text{methoxyl}}$, $\delta^2\text{H}_{\text{fatty-acids}}$ ($n\text{-alkanoic acids}$) and $\delta^2\text{H}_{n\text{-alkanes}}$ in order to establish a multi-proxy stable isotope record for the Late Quaternary in equatorial East Africa (Figure 5). Pollen results for the same sequence were previously presented by Schöler et al. (2012). The coupled $\delta^2\text{H}_{n\text{-alkanes}}\text{-}\delta^{18}\text{O}_{\text{sugars}}$ paleohygrometer approach was used to reconstruct mean RH values during day-time (RH_{HD}) and source water isotope composition ($\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$). The $\delta^2\text{H}_{\text{fatty-acids}}$ and $\delta^2\text{H}_{n\text{-alkanes}}$ records of Maundi were compared to the $\delta^2\text{H}_{\text{leaf-wax}}$ records of Lake Challa, Lake Tanganyika and Lake Malawi (Tierney et al., 2008; Tierney et al., 2011; Konecky et al., 2011). They all reveal the same trends, i.e. more negative values during the African Humid Period. At the same time, differences regarding the distinct patterns, amplitude and timing of events are also observed (Figure 5).

A straightforward interpretation of these climate proxies is complicated by the numerous possibly influencing factors on sedimentary fatty acids and *n*-alkanes in East Africa (i.e. moisture source and transport history, precipitation amount and evapotranspirative enrichment). Therefore, the coupled $\delta^2\text{H}_{n\text{-alkanes}}-\delta^{18}\text{O}_{\text{sugars}}$ paleohygrometer approach was used to derive quantitative paleoclimate information, potentially overcoming these limitation. Reconstructed RH_D values for Maundi range from 29 to 81% (Figure 6). The most pronounced minimum during ~70 to 60 ka is with dating uncertainties corresponding to a pronounced famous drought period leading to low lake levels of Lake Malawi ('megadroughts' according to Scholz et al. (2007) and Lake Challa (Moernaut et al., 2010). The two minima during the Last Glacial Maximum also coincide with low lake levels of Lake Tanganyika (Gasse et al., 1989; McGlue et al., 2007), Lake Viktoria (Talbot and Livingstone, 1989) and Lake Challa (Verschuren et al., 2009; Moernaut et al., 2010). The shift towards more humid conditions during the Late Glacial seen in the Maundi RH_D record reflects the beginning of the African Humid Period, as suggested by raising equatorial East African lake levels (Gasse, 2000; Junginger et al., 2014), and enhanced rainfall derived from model results (Otto-Bliesner et al., 2014).

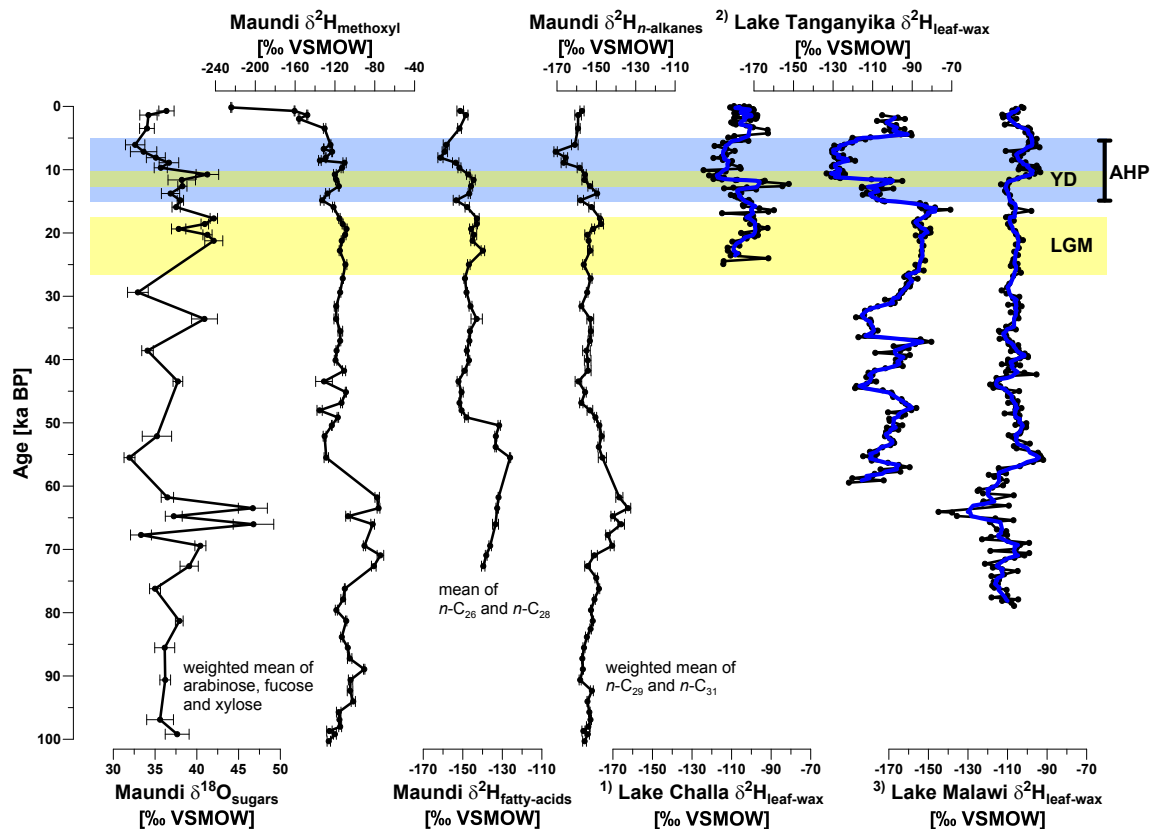


Figure 5: Maundi multi-proxy (biomarker) isotope records ($\delta^{18}\text{O}_{\text{sugars}}$, $\delta^2\text{H}_{n\text{-alkanes}}$, $\delta^2\text{H}_{\text{fatty-acids}}$, $\delta^2\text{H}_{\text{methoxyl}}$) along with Lake Challa (Tierney et al., 2011), Lake Tanganyika (Tierney et al., 2008) and Lake Malawi (Konecky et al., 2011) leaf wax $\delta^2\text{H}$ records (as presented in Hepp et al., 2017). LGM = Last Glacial Maximum, YD = Younger Dryas, AHP = African Humid Period.

Overall the Maundi RH_D record is in agreement with the climate reconstruction based on the Maundi pollen results (Schüler et al., 2012). In addition to the regional moisture availability, a local effect is suggested to influence the Maundi RH_D values. This local effect

concerns the intensification of the trade wind inversion, which affects the diurnal atmospheric circulation on the Southern slopes of Mt. Kilimanjaro (Pepin et al., 2010). This can help to explain why the Maundi RH_D record does not show noticeably humid conditions during the Early Holocene, while high lake levels during the Early Holocene document the African Humid Period until ~5 ka as a period with moisture availability in East African region after the interruption caused by the Younger Dryas (Junginger et al., 2014).

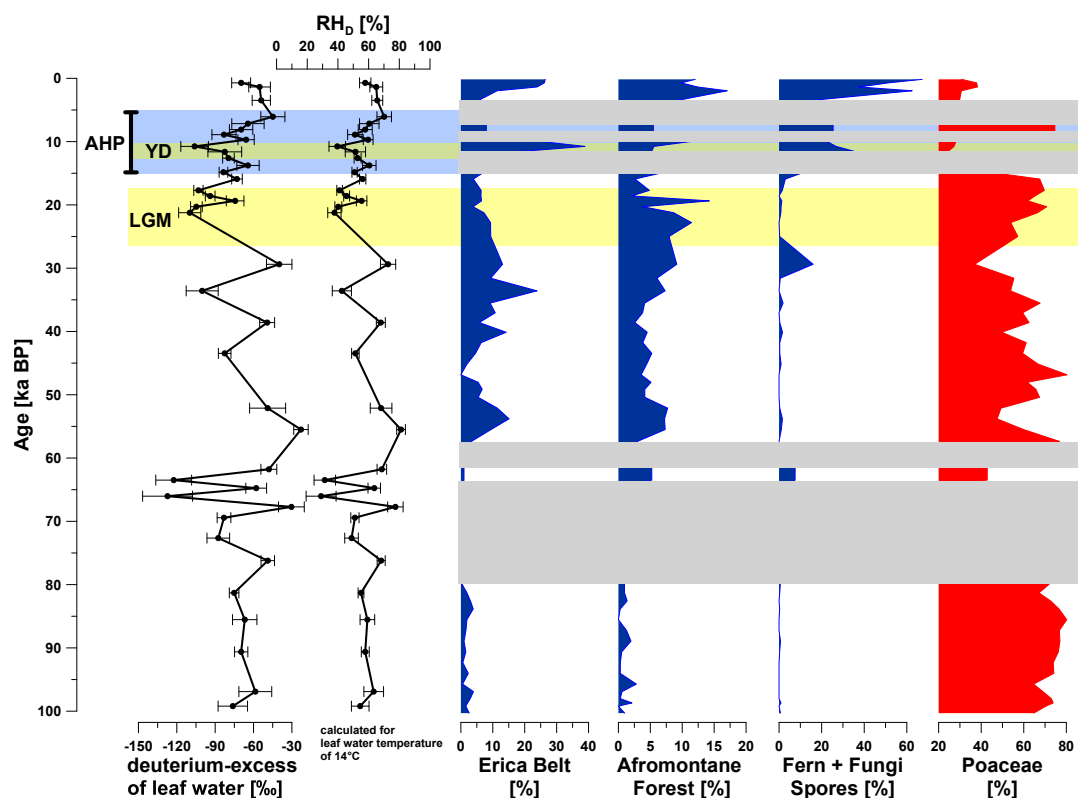


Figure 6: Maundi deuterium-excess of leaf water and reconstructed RH during day-time (RH_D) records, along with selected pollen results from the Maundi loess-like paleosol sequence (Schüler et al., 2012), as modified from Hepp et al. (2017). LGM = Last Glacial Maximum, YD = Younger Dryas, AHP = African Humid Period.

Finally, the highly significant correlation between reconstructed deuterium-excess of leaf water and $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ (Figure 7) reveals that a long-term amount effect cannot explain the pattern of the Maundi $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ records. In modern precipitation, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for East African regions on a seasonal timescale can be described (Rozanski et al., 1996). However, no clear relationship between $\delta^2\text{H}_{\text{precipitation}}$, $\delta^{18}\text{O}_{\text{precipitation}}$ and precipitation amount is observed on longer (at least inter-annual) time scales (Rozanski et al., 1996; Sundqvist et al., 2013). Most likely effects on local and regional moisture recycling, and therefore the expansion, shrinking (or complete collapse) of montane rainforest on the Southeastern slopes of Mt. Kilimanjaro and changes in regional vegetation cover have to be taken into account for understanding Maundi $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ variations.

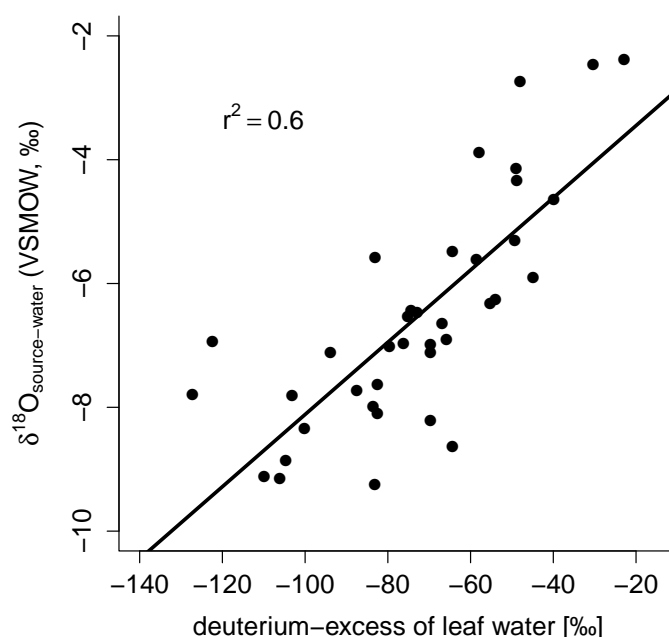


Figure 7: Scatterplot of reconstructed Maundi $\delta^{18}\text{O}_{\text{source-water}}$ versus deuterium-excess of leaf water, revealing a positive correlation with an r^2 of 0.6 (using the data as presented in Tab. 1 of Hepp et al., 2017).

4.4 Source identification of terrestrial versus aquatic sugars in lacustrine systems (manuscript 4)

For a successful application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach to lacustrine archives, the identification of the sedimentary organic matter source, and more specifically the identification of the source of specific biomarkers is essential. With regard to n -alkane biomarkers, source identification is mainly based on chain-lengths. Long-chain lipids (n -alkanes, n -alkanols and n -alkanoic acids) are usually interpreted as derived from terrestrial plants because they occur abundantly in epicuticular leaf waxes of higher vascular plants (Eglinton and Hamilton, 1967). By contrast, mid- and short chain lipids are usually associated with submerged aquatic macrophytes (Ficken et al., 2000) or algae. Yet, this source assignment is increasingly challenged (e.g. Hepp et al., 2015; Aichner et al., 2018) and needs careful consideration in every case study. For the sugar biomarkers arabinose, fucose and xylose, a lacustrine sedimentary source identification is presented in this manuscript for the first time.

Sugar biomarkers were extracted from different terrestrial and aquatic plants as well as from various algae species. While vascular plants are characterized by high amounts of arabinose and xylose, algae yielded higher concentrations of fucose (both based on relative sugar biomarker abundances; Figure 8A). In combination with data compiled from the literature this suggests that the ratio of fuc/(ara + xyl) can serve as an additional proxy for differentiating between aquatic versus terrestrial lacustrine sedimentary organic matter input. When additionally taking into account relative sugar biomarker abundances from

soils and sediments (Figure 8B), the ratio of (fuc + xyl)/ara seems to be a helpful proxy for distinguishing aquatic versus terrestrial input in lacustrine archives, too.

Overall, this compilation suggests that sugar biomarkers can serve as valuable complementary proxy for sedimentary source identification and that fucose and xylose can often be related to aquatic sources, whereas arabinose can often be attributed to terrestrial origin. The latter likely holds true for the Lake Gemündener Maar sediments (Figure 8B).

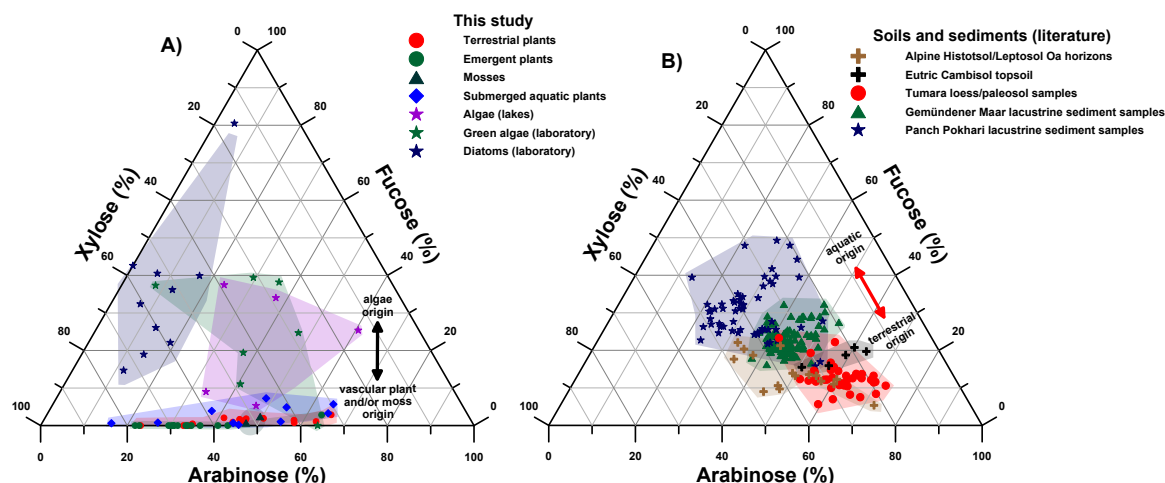


Figure 8: Ternary diagrams illustrating the relative abundances of arabinose, fucose and xylose for the analyzed samples presented in manuscript 4 (Hepp et al., 2016) (A), as well as for soil and sediment data compiled from the literature, i.e. alpine soils (Prietz et al., 2013), topsoil samples (Bock et al., 2007), loess/paleosol samples (Zech et al., 2013) and lacustrine sediments (Zech et al., 2014a) (B). Figure modified from Hepp et al. (2016).

4.5 Application of the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach to the Gemündener Maar sedimentary record (manuscript 5)

The above-presented source identification study of terrestrial versus aquatic sugars in lacustrine systems (manuscript 4) provides the basis for applying the coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach to the Late Glacial-Early Holocene Lake Gemündener Maar sediment archive. The Lake is located in the Western Eifel region (50°10'39.853"N, 6°50'12.912"E; 407 m a.s.l.), Germany, within the ancient volcanic field (Sirocko et al., 2013). The weighted mean $\delta^2\text{H}$ of the alkanes $n\text{-C}_{27}$ and $n\text{-C}_{29}$ as well as $\delta^{18}\text{O}_{\text{arabinose}}$ are used to derive RH day-time and vegetation period (RH_{dv}), because they are considered to record the past $\delta^2\text{H}_{\text{leaf-water}}$ and $\delta^{18}\text{O}_{\text{leaf-water}}$.

Interestingly, the results suggest that the Younger Dryas was not uniformly dry but two-phased with regard to RH. The first phase is characterised by RH_{dv} values similar to the Allerød level; only the end of the Younger Dryas is characterised by drier climatic conditions (Figure 9). This contradicts earlier results suggesting (i) continuously dry conditions throughout the Younger Dryas (Rach et al., 2014) and (ii) a two-phasing with a dry and

cold first period that is followed by increasing wetness and higher temperatures during the second Younger Dryas phase (Brauer et al., 1999). Moreover, the Lake Gemündener Maar RH record reveals quite high variability during the Early Holocene, compared to the Younger Dryas and the Allerød sections. So far, it is uncertain how strong the solar activity influenced this unexpected finding in Late Glacial-Early Holocene RH history over Central Europe.

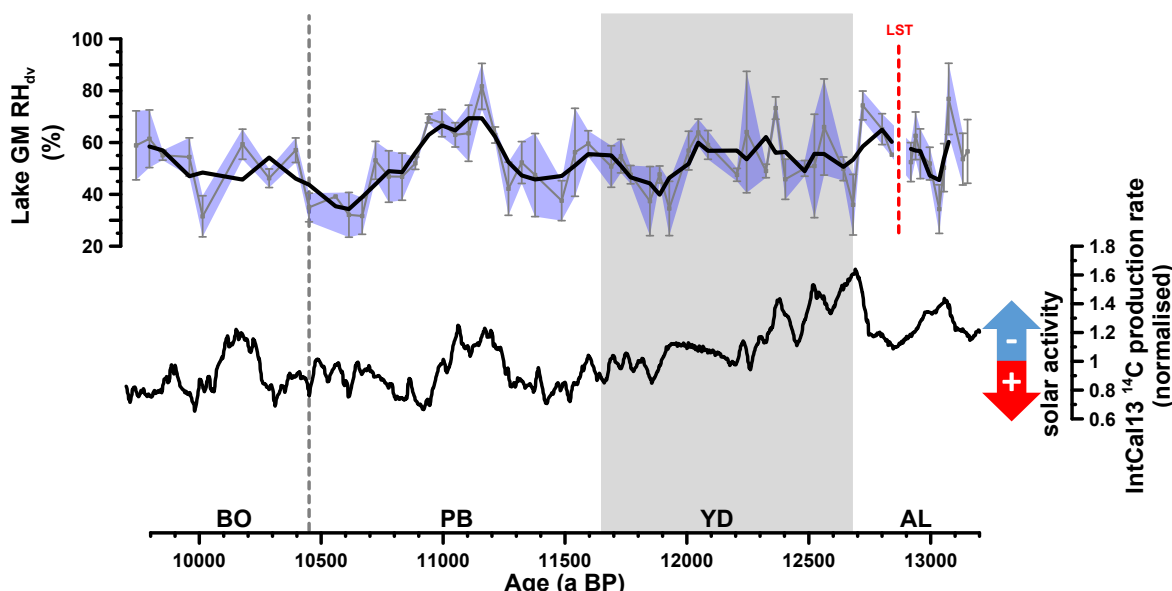


Figure 9: A) Lake Gemündener Maar relative humidity record during day-time and vegetation period (RH_{dv}) along with B) the IntCal 13 ^{14}C production rate (Muscheler et al., 2014), which can be interpreted as solar activity proxy (Stuiver and Braziunas, 1988). Bold line in RH_{dv} plot = 3 point running mean; Error bars and shaded area represents uncertainties associated with $\delta^2H_{n-alkane}$ and $\delta^{18}O_{sugar}$ measurements. AL = Allerød, LST = Laacher See Tephra, YD = Younger Dryas, PB = Preboreal, BO = Boreal. Figure modified from Hepp et al. (2019a).

4.6 Application of the coupled $\delta^2H_{n-alkane}$ - $\delta^{18}O_{sugar}$ approach to the Lake Bergsee sedimentary record (manuscript 6)

The second application of the coupled $\delta^2H_{n-alkane}$ - $\delta^{18}O_{sugar}$ approach to a lake sedimentary archive was conducted on samples from Lake Bergsee, Southern Black Forest, Germany (7°56'11"E, 47°34'20"N; 382 m a.s.l.; Becker et al., 2006). The investigated core section covers, like the Lake Gemündener Maar study, the Late Glacial to Early Holocene transition, i.e. a time span between 16,000 to 10,750 a cal BP. As highlighted by Hepp et al. (2015) (section 4.4 and manuscript 4) and Hepp et al. (2019a) (section 4.5 and manuscript 5), the biomarker source identification is essential for interpreting the compound-specific isotope results. For the biomarker source identification, n -alkane and sugar biomarker pattern were analyzed in detail and, for potentially coupling $\delta^2H_{n-alkane}$ with $\delta^{18}O_{sugar}$ results, alkanes with the chain length n -C₂₃, n -C₂₅, n -C₂₇, n -C₂₉ and n -C₃₁ as well as the sugars arabinose, fucose and xylose were analyzed for their δ^2H and $\delta^{18}O$ isotope signatures, respectively.

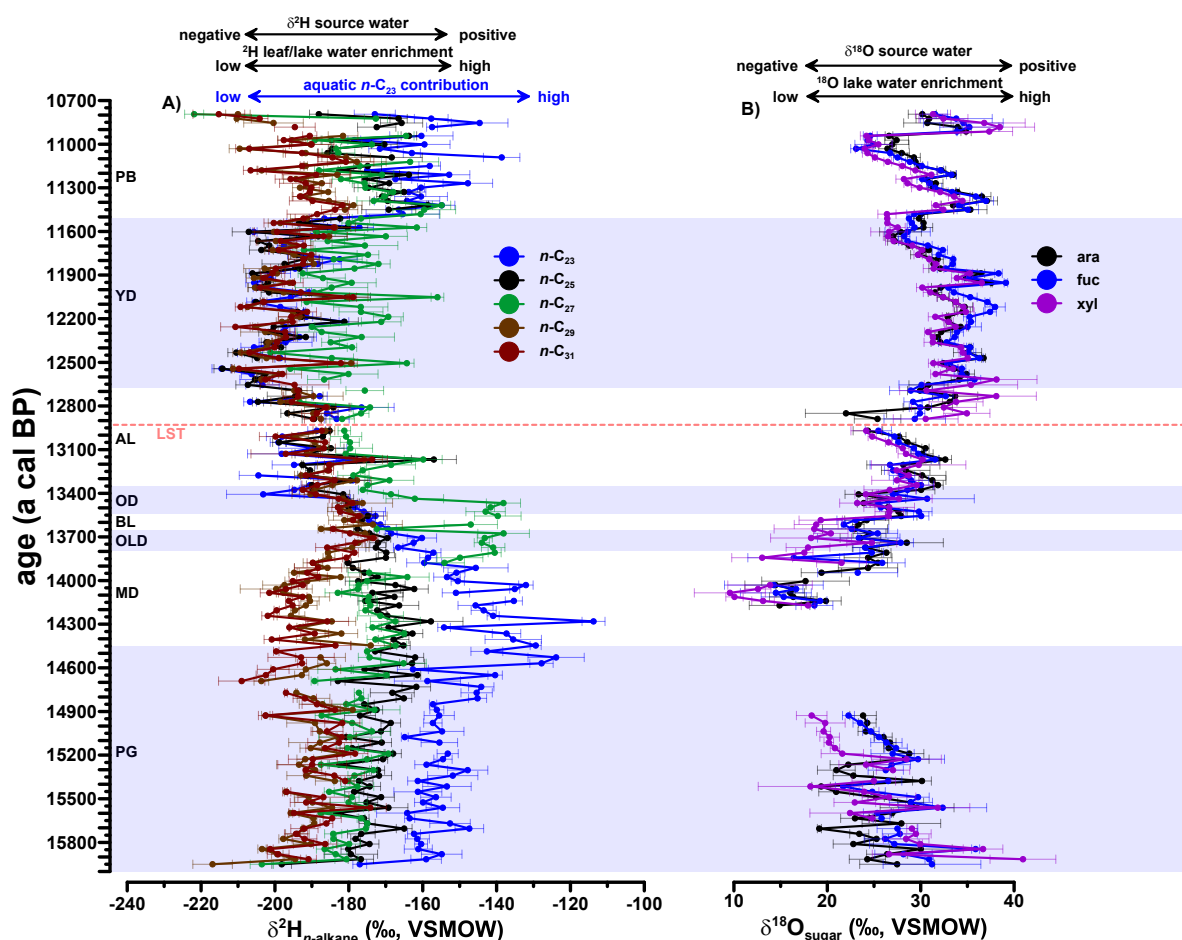


Figure 10: A) $\delta^2\text{H}_{n\text{-alkane}}$ records ($n\text{-C}_{23}$, $n\text{-C}_{25}$, $n\text{-C}_{27}$, $n\text{-C}_{29}$ and $n\text{-C}_{31}$) and B) $\delta^{18}\text{O}_{\text{sugar}}$ records (arabinose, fucose, xylose) of Lake Bergsee. Background colors show time periods (according to Litt et al., 2001): PB = Preboreal, YD = Younger Dryas, AL = Allerød, OD = Older Dryas, BL = Bølling, OLD = Oldest Dryas, MD = Meiendorf, PG = Pleniglacial. LST = Laacher See Tephra.

An unambiguous bulk source determination turned out to be not possible for Lake Bergsee based on the bulk results. Regarding the n -alkane biomarkers, however, it can be assumed that the long-chain homologues ($n\text{-C}_{27}$, $n\text{-C}_{29}$ and $n\text{-C}_{31}$) originate from terrestrial sources (Eglinton and Hamilton, 1967), i.e. from leaf waxes of higher terrestrial plants grown in the Lake Bergsee catchment. The short-chain compound ($n\text{-C}_{23}$) reflect most likely a mixture between the input from submerged aquatic organisms (e.g. Ficken et al., 2000) and from terrestrial plants, as shown also by Aichner et al. (2018) for a lacustrine record from Poland. Sedimentary sugars are interpreted to be primarily aquatic-derived, based on the sugar ratios developed from manuscript 4 (Figure 8). Accordingly, $\delta^{18}\text{O}_{\text{sugar}}$ (arabinose, xylose and fucose) are presumably good $\delta^{18}\text{O}_{\text{lake-water}}$ recorders, while $\delta^2\text{H}_{n\text{-alkane}}$ values from long-chain n -alkane ($n\text{-C}_{27}$, $n\text{-C}_{29}$ and $n\text{-C}_{31}$) should reflect paleo $\delta^2\text{H}_{\text{leaf-water}}$.

The origin of the n -alkane and sugar biomarkers becomes also obvious when describing the biomarker-based isotope records (Figure 10). When the $n\text{-C}_{29}$ and $n\text{-C}_{31}$ alkanes originate from grasses (e.g. *Poaceae*) and $n\text{-C}_{27}$ from trees (e.g. *Betula*), in average +17‰ more positive $n\text{-C}_{27}$ $\delta^2\text{H}$ values can be explained with the fact that n -alkanes from grasses are typically less sensitive leaf water enrichment recorders (McInerney et al., 2011; Kahmen et al., 2013a). The $n\text{-C}_{25}$ $\delta^2\text{H}$ record seems to be a mixture between tree (*Betula*)

and grass (*Poaceae*) input, because the values are close to $n\text{-C}_{27}$ during the Pleniglacial, Meindorf and the Preboreal, while during Oldest Dryas, Bølling, Older Dryas, Allerød and Younger Dryas the $n\text{-C}_{25}$ $\delta^2\text{H}$ record resemble the $n\text{-C}_{29}$, $n\text{-C}_{31}$ ones. For long-chain n -alkanes, often an $\varepsilon_{\text{bio}}^2$ factor of -160‰ is assumed, based on findings from Sachse et al. (2006) and Sessions et al. (1999). For $n\text{-C}_{23}$ $\delta^2\text{H}$ data from *Potamogeton* and surface sediments, however, a smaller fractionation factor during biosynthesis of -82 to -88‰ is suggested (Aichner et al., 2010). Offsets between Lake Bergsee $n\text{-C}_{23}$ $\delta^2\text{H}$ and $n\text{-C}_{27}$, $n\text{-C}_{29}$, $n\text{-C}_{31}$ $\delta^2\text{H}$ records could therefore result from variable aquatic contribution of $n\text{-C}_{23}$. The main influencing factors on n -alkane $\delta^2\text{H}$ are highlighted in Figure 10A.

The Lake Bergsee $\delta^{18}\text{O}_{\text{sugar}}$ records can be interpreted in terms of reflecting changes in (i) $\delta^{18}\text{O}$ of source water (\sim local precipitation) and (ii) ^{18}O lake water enrichment (as illustrated in Figure 10B). This can explain why the $\delta^{18}\text{O}_{\text{sugar}}$ record of Lake Bergsee reveals opposite trends and a much higher amplitude compared to precipitation records based on carbonate $\delta^{18}\text{O}$ (Mayer and Schwark, 1999; Wurth et al., 2004). Focusing on the Younger Dryas-Preboreal transition, a shift of $\sim 5\text{‰}$ is obvious in $\delta^{18}\text{O}_{\text{sugar}}$ record, based on average values for both periods. This is well in agreement with a reconstructed $\delta^{18}\text{O}_{\text{lake-water}}$ shift of around 6‰ from Lake Gosciadz (Rozanski et al., 2010).

Due to the mixed origin of $n\text{-C}_{23}$ in Lake Bergsee sediments (= mixture between aquatic and terrestrial sources) a coupling between $\delta^2\text{H}_{n\text{-alkane}}$ ($n\text{-C}_{23}$) and $\delta^{18}\text{O}_{\text{sugar}}$ according to Hepp et al. (2015) was not possible. The large potential of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach for disentangling lake or leaf water enrichments from source water changes could therefore not be utilized in this study. Moreover, our results are in line with other publications emphasising that caution has to be taken when applying the classical n -alkane chain-length interpretation to lacustrine archives (Hepp et al., 2015; Duan et al., 2016; Liu and Liu, 2016; Rao et al., 2016).

5 Conclusions

The following conclusions can be drawn from the results and discussions presented in this thesis dealing with the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach:

- The analysis of the leaf material from the climate chamber experiment shows that $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ are well correlated with $\delta^2\text{H}_{\text{leaf-water}}$, $\delta^{18}\text{O}_{\text{leaf-water}}$ ($r^2 = 0.45$ and 0.85 , respectively, $p < 0.001$, $n = 24$). Moreover, RH_{air} can be robustly reconstructed based on measured $\delta^2\text{H}_{\text{leaf-water}}$, $\delta^{18}\text{O}_{\text{leaf-water}}$ values as well as on $\delta^2\text{H}_{n\text{-alkane}}$, $\delta^{18}\text{O}_{\text{sugar}}$ -derived leaf water isotope composition by using a simplified Craig-Gordon model. This highlights the large potential of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach. From the topsoil transect study, it can be concluded that such an $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach allows the reconstruction of $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and RH_{MDV} values. However, also systematical offsets between biomarker-based (reconstructed) $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and RH_{MDV} values compared to precipitation $\delta^2\text{H}_{\text{GIPR,OIPC}}$, $\delta^{18}\text{O}_{\text{GIPR,OIPC}}$ and climate station RH_{MDV} values, respectively, are observed. Thus, both studies imply that the uncertainty of reconstructing $\delta^2\text{H}_{\text{leaf-water}}$ based on $\delta^2\text{H}_{n\text{-alkane}}$ values (which is also present in data compiled from literature, see section 3.1.1) represents one clear limitation of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach regarding the reconstruction of rather small variability in $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and RH records.

- The application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach to the terrestrial climate archive Maundi was still successful in terms of establishing $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and RH_D records for the last ~100 ka for the East African region. The results indicate that leaf water enrichment can mask changes in precipitation isotope composition, both incorporated in $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$. The coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach is shown to have the potential to disentangle between those signals. A strong relationship between biomarker-based $\delta^2\text{H}_{\text{source-water}}$, $\delta^{18}\text{O}_{\text{source-water}}$ and RH_D furthermore points against the presence of an amount effect on $\delta^2\text{H}_{\text{precipitation}}$ and $\delta^{18}\text{O}_{\text{precipitation}}$ isotope composition on long-time scales.
- The source identification study of terrestrial versus aquatic sugar biomarkers in lake sediments shows in general that the relative abundances of arabinose, fucose and xylose can be used to distinguish between algae and terrestrial plant sources. Arabinose from Late Glacial-Early Holocene Lake Gemündener Maar sediments is primarily of terrestrial origin, whereas fucose and xylose stem predominately from aquatic sources.
- The $\delta^{18}\text{O}_{\text{arabinose}}$ and terrestrial $\delta^2\text{H}_{n\text{-alkane}}$ ($n\text{-C}_{27}$, $n\text{-C}_{29}$) results derived from Lake Gemündener Maar sediments were used in a coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach. The established RH_{dv} record challenges that the Younger Dryas was characterized by overall dry climatic conditions. There was rather a relatively wet phase at the beginning of the Younger Dryas, which is followed by a drier late Younger Dryas. Furthermore, large RH_{dv} changes during the Early Holocene are observed, which are even more pronounced than the variations during the Allerød-Younger Dryas transition. Unlike the Lake Gemündener Maar study, the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach could not be applied to Lake Bergsee Late Glacial-Early Holocene sediments. While the long-chain $n\text{-alkanes}$ can be attributed to terrestrial sources and the sugars to primarily aquatic sources, $n\text{-C}_{23}$ is most likely a mixture of both origins. A clear differentiation between terrestrial or aquatic origins of the $n\text{-alkane}$ and sugar biomarkers seems to be fundamental for a successful application of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach to lacustrine archives.

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Contributions to the included manuscripts

The presented cumulative thesis is comprised of 6 manuscripts prepared with the contributions from all co-authors. A record of the specific author contribution is given below:

Manuscript 1: Validation of a coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach based on a climate chamber experiment

Johannes Hepp:	laboratory work ($\delta^{18}\text{O}$ of sugars), modeling, discussion of results, manuscript preparation, publication handling (60%)
Bruno Glaser:	discussion of results, comments on the manuscript (3%)
Dieter Juchelka:	comments on the manuscript (2%)
Christoph Mayr:	climate chamber experiment, discussion of results, comments on the manuscript (7%)
Kazimierz Rozanski:	discussion of results, comments on the manuscript (5%)
Imke Kathrin Schäfer:	laboratory work ($\delta^2\text{H}$ of n -alkanes), data preparation and evaluation, comments on the manuscript (5%)
Willibald Stichler:	climate chamber experiment, comments on the manuscript (2%)
Mario Tuthorn:	laboratory work ($\delta^{18}\text{O}$ of sugars), data preparation and evaluation, discussion of results, comments on the manuscript (7%)
Roland Zech:	laboratory work ($\delta^2\text{H}$ of n -alkanes), discussion of results, comments on the manuscript (2%)
Michael Zech:	discussion of results, comments on the manuscript (7%)

Manuscript 2: Evaluation of bacterial glycerol dialkyl glycerol tetraether and ^2H - ^{18}O biomarker proxies along a Central European topsoil transect

Johannes Hepp:	laboratory work ($\delta^{18}\text{O}$ of sugars and pH), data evaluation and preparation, performed coupled ^2H - ^{18}O biomarker approach modelling, discussion of results, manuscript preparation, publication handling (40%)
Imke Kathrin Schäfer:	data evaluation and preparation ($\delta^2\text{H}$ of <i>n</i> -alkanes and GDGT's), discussion of results, manuscript preparation (30%)
Verena Lanny:	field work, laboratory work ($\delta^2\text{H}$ of <i>n</i> -alkanes and GDGT's), discussion of results, comments on the manuscript (6%)
Jörg Franke:	statistical analysis, discussion of results, comments on the manuscript (2%)
Marcel Bliedtner:	assistance with laboratory work ($\delta^2\text{H}$ of <i>n</i> -alkanes and GDGT's), discussion of results, comments on the manuscript (2%)
Kazimierz Rozanski:	assistance with coupled ^2H - ^{18}O biomarker approach modelling, discussion of the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results, comments on the manuscript (5%)
Bruno Glaser:	discussion of the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results, comments on the manuscript (2%)
Michael Zech:	discussion of the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results, comments on the manuscript (3%)
Timothy Eglinton:	discussion of the GDGT results, comments on the manuscript (2%)
Roland Zech:	study design, discussion of results, comments on the manuscript (8%)

Manuscript 3: Late Quaternary relative humidity changes from Mt. Kilimanjaro, based on a coupled ^2H - ^{18}O biomarker paleohygrometer approach

Johannes Hepp:	data evaluation and compilation, discussion of results, manuscript preparation, publication handling (51%)
Roland Zech:	laboratory work ($\delta^2\text{H}$ of <i>n</i> -alkanes and fatty acids), data preparation, discussion of the results, comments on the manuscript (10%)
Kazimierz Rozanski:	assistance with modelling, comments on the manuscript (7%)
Mario Tuthorn:	laboratory work ($\delta^{18}\text{O}$ of sugars), discussion of the results, comments on the manuscript (7%)
Bruno Glaser:	discussion of results, comments on the manuscript (3%)
Markus Greule:	laboratory work ($\delta^2\text{H}$ of methoxyl groups), data preparation, discussion of results, comments on the manuscript (3%)
Frank Keppler:	laboratory work ($\delta^2\text{H}$ of methoxyl groups), discussion of results, comments on the manuscript (3%)
Yongsong Huang:	laboratory work ($\delta^2\text{H}$ of <i>n</i> -alkanes and fatty acids), comments on the manuscript (3%)
Wolfgang Zech:	field work, comments on the manuscript (3%)
Michael Zech:	laboratory work ($\delta^{18}\text{O}$ of sugars), data preparation, discussion of the results, comments on the manuscript (10%)

Manuscript 4: A sugar biomarker proxy for assessing terrestrial versus aquatic sedimentary input

Johannes Hepp:	field work (recent plant samples), laboratory work (sugar measurements of recent plant, sediment and algae samples), data preparation and evaluation of recent plant, sediment and algae samples, discussion of results, manuscript preparation, publication handling (65%)
Max Rabus:	laboratory work (algae cultures), discussion of results, comments on the manuscript (5%)
Tobias Anhäuser:	field work (recent plant and sediment), comments on the manuscript (3%)
Tobias Bromm:	Lake Gemündener Maar (GM) sediment samples, laboratory work (sugar measurements of lake GM sediment samples), data preparation and evaluation of lake GM sediment samples, comments on the manuscript (4%)
Christian Laforsch:	laboratory work (algae cultures), discussion of results, comments on the manuscript (5%)
Frank Sirocko:	Lake GM sediment samples, comments on the manuscript (3%)
Bruno Glaser:	discussion of results, comments on the manuscript (3%)
Michael Zech:	field work (recent plant and sediment sample samples), Lake GM sediment samples, assistance with data preparation and evaluation, discussion of results, comments on the manuscript (12%)

Manuscript 5: How dry was the Younger Dryas? Evidence from a coupled $\delta^2\text{H}$ - $\delta^{18}\text{O}$ biomarker paleohygrometer, applied to the Lake Gemündener Maar sediments, Western Eifel, Germany

Johannes Hepp:	data evaluation and compilation, modelling, discussion of results, co-writing an earlier version of the manuscript, manuscript preparation of the actual version, publication handling (52%)
Lorenz Wüthrich:	laboratory work ($\delta^2\text{H}$ of <i>n</i> -alkanes), data preparation and evaluation, discussion of results, manuscript preparation of an earlier version of the manuscript, comments on the actual version of the manuscript (15%)
Tobias Bromm:	laboratory work ($\delta^{18}\text{O}$ of sugars), data preparation and evaluation, discussion of results, comments on the manuscript (3%)
Marcel Bliedtner:	laboratory work ($\delta^2\text{H}$ of <i>n</i> -alkanes), data preparation and evaluation, discussion of results, comments on the manuscript (3%)
Imke Kathrin Schäfer:	laboratory work ($\delta^2\text{H}$ of <i>n</i> -alkanes), data preparation and evaluation, discussion of results, comments on the manuscript (3%)
Bruno Glaser:	discussion of results, comments on the manuscript (2%)
Kazimierz Rozanski:	assistance with modelling, discussion of results, comments on the manuscript (6%)
Frank Sirocko:	lake coring, chronology, discussion of results, comments on the manuscript (3%)
Roland Zech:	discussion of results, co-writing an earlier version of the manuscript (6%)
Michael Zech:	core sampling, assistance with laboratory work, discussion of results, co-writing an earlier version of the manuscript, comments on the actual version of the manuscript (7%)

Manuscript 6: Late Glacial to Early Holocene $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}$ records from Lake Bergsee, Black Forest, Germany – potential and limitations

Johannes Hepp:	core sampling, laboratory work (bulk measurements, sugar and $\delta^{18}\text{O}$ of sugars, n -alkanes and $\delta^2\text{H}$ of n -alkanes), data preparation, evaluation and compilation, discussion of results, manuscript preparation (60%)
Lucas Kämpf:	core sampling, laboratory work (sugars and $\delta^{18}\text{O}$ of sugars), discussion of results, comments on the manuscript (5%)
Damien Rius:	lake coring, chronology, pollen results, discussion of the results, comments on the manuscript (5%)
Mario Tuthorn:	laboratory work (bulk measurements, sugar and $\delta^{18}\text{O}$ of sugars), discussion of results, comments on the manuscript (5%)
Lucas Bittner:	laboratory work ($\delta^{18}\text{O}$ of sugar), data evaluation, discussion of results, comments on the manuscript (3%)
Laurent Millet:	lake coring, chronology, pollen results, discussion of the results, comments on the manuscript (3%)
Fanny Dupart-Oualid:	pollen analysis, discussion of results, comments on the manuscript (2%)
Bruno Glaser:	discussion of the results, comments of the manuscript (2%)
Michael Zech:	core sampling, assistance with laboratory work ($\delta^{18}\text{O}$ of sugars and $\delta^2\text{H}$ of n -alkanes), discussion of results, comments on the manuscript (15%)

Included manuscripts

A. Manuscript 1: Hepp et al. (2019c)

submitted to *Biogeosciences*, published in *Biogeosciences Discussion*

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Validation of a coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach based on a climate chamber experiment

Johannes Hepp^{a,b,1,*,#}, Bruno Glaser^b, Dieter Juchelka^c, Christoph Mayr^{d,e,2}, Kazimierz Rozanski^f, Imke Kathrin Schäfer^g, Willibald Stichler^h, Mario Tuthorn^{c,3}, Roland Zech^{g,i,4}, Michael Zech^{b,j,5,#}

^aChair of Geomorphology and BayCEER, University of Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth, Germany

^bInstitute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin-Luther-University Halle-Wittenberg, Von-Seckendorff-Platz 3, D-06120 Halle (Saale), Germany

^cThermo Fisher Scientific, Hanna-Kunath-Str. 11, D-28199 Bremen, Germany

^dInstitute of Geography, Friedrich-Alexander-University Erlangen-Nürnberg, Wetterkreuz 15, D-91058 Erlangen, Germany

^eGeoBio-Center & Earth and Environmental Sciences, Ludwig-Maximilian University Munich, Richard-Wagner-Str. 10, D-80333 München, Germany

^fFaculty of Physics and Applied Computer Science, AGH University of Science and Technology, Al. Mickiewicza 30, PL-30-059 Kraków, Poland

^gInstitute of Geography and Oeschger Centre for Climate Research, University of Bern, Hallerstrasse 12, CH-3012 Bern, Switzerland

^hHelmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany

ⁱInstitute of Geography, Chair of Physical Geography, Friedrich-Schiller University of Jena, Löbdergraben 32, D-07743 Jena, Germany

^jInstitute of Geography, Heisenberg Chair of Physical Geography with focus on paleoenvironmental research, Technical University of Dresden, Helmholtzstrasse 10, D-01062 Dresden, Germany

*corresponding author: johannes-hepp@gmx.de

#all other co-authors are listed alphabetically

¹Present address: Chair of Geomorphology and BayCEER, University of Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth, Germany

²Present address: Institute of Geography, Friedrich-Alexander-University Erlangen-Nürnberg, Wetterkreuz 15, D-91058 Erlangen, Germany

³Present address: Thermo Fisher Scientific, Hanna-Kunath-Str. 11, D-28199 Bremen, Germany

⁴Present address: Institute of Geography, Chair of Physical Geography, Friedrich-Schiller University of Jena, Löbdergraben 32, D-07743 Jena, Germany

⁵Present address: Institute of Geography, Heisenberg Chair of Physical Geography with focus on paleoenvironmental research, Technical University of Dresden, Helmholtzstrasse 10, D-01062 Dresden, Germany

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Keywords

hydrogen stable isotopes, oxygen stable isotopes, hemicellulose sugars, leaf waxes, leaf water enrichment, deuterium-excess, relative humidity

Abstract

The hydrogen isotopic composition of leaf wax-derived biomarkers, e.g. long chain n -alkanes ($\delta^2\text{H}_{n\text{-alkane}}$), is widely applied in paleoclimatology research. However, a direct reconstruction of the isotopic composition of paleoprecipitation based on $\delta^2\text{H}_{n\text{-alkane}}$ alone can be challenging due to the overprint of the source water isotopic signal by leaf-water enrichment. The coupling of $\delta^2\text{H}_{n\text{-alkane}}$ with $\delta^{18}\text{O}$ of hemicellulose-derived sugars ($\delta^{18}\text{O}_{\text{sugar}}$) has the potential to disentangle this effect and additionally allow relative humidity reconstructions. Here, we present $\delta^2\text{H}_{n\text{-alkane}}$ as well as $\delta^{18}\text{O}_{\text{sugar}}$ results obtained from leaves of the plant species *Eucalyptus globulus*, *Vicia faba* var. *minor* and *Brassica oleracea* var. *medullosa*, which were grown under controlled conditions. We addressed the questions (i) do $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ values allow precise reconstructions of leaf water isotope composition, (ii) how accurately does the reconstructed leaf-water-isotope composition enables relative humidity (RH) reconstruction in which the plants grew, and (iii) does the coupling of $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ enable a robust source water calculation?

For all investigated species, the alkane $n\text{-C}_{29}$ was most abundant and therefore used for compound-specific $\delta^2\text{H}$ measurements. For *Vicia faba*, additionally the $\delta^2\text{H}$ values of $n\text{-C}_{31}$ could be evaluated robustly. With regard to hemicellulose-derived monosaccharides, arabinose and xylose were most abundant and their $\delta^{18}\text{O}$ values were therefore used to calculate weighted mean leaf $\delta^{18}\text{O}_{\text{sugar}}$ values. Both $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ yielded significant correlations with $\delta^2\text{H}_{\text{leaf-water}}$ and $\delta^{18}\text{O}_{\text{leaf-water}}$, respectively ($r^2 = 0.45$ and 0.85 , respectively; $p < 0.001$, $n = 24$). Mean fractionation factors between biomarkers and leaf water were found to be -156‰ (ranging from -133 to -192‰) for $\epsilon_{n\text{-alkane/leaf-water}}$ and $+27.3\text{‰}$ (ranging from $+23.0$ to 32.3‰) for $\epsilon_{\text{sugar/leaf-water}}$, respectively. Using rearranged Craig-Gordon equations with either T_{air} or T_{leaf} and measured $\delta^2\text{H}_{\text{leaf-water}}$ or $\delta^{18}\text{O}_{\text{leaf-water}}$ as input variables, we furthermore modeled climate chamber RH_{air} and RH_{leaf} values. Modelled RH_{air} values, from the more simplified Craig-Gordon model, turned out to be most accurate and correlate highly significantly with measured RH_{air} values ($R^2 = 0.84$, $p < 0.001$; $\text{RMSE} = 6\%$). When combining $\delta^2\text{H}_{\text{leaf-water}}$ and $\delta^{18}\text{O}_{\text{leaf-water}}$ values that are calculated from the alkane and sugar biomarkers instead of actually measured $\delta^2\text{H}_{\text{leaf-water}}$ and $\delta^{18}\text{O}_{\text{leaf-water}}$ as input variables, the correlation of modelled RH_{air} values with measured RH_{air} values is getting worse, but is still highly significant with $R^2 = 0.54$, $p < 0.001$; $\text{RMSE} = 10\%$. This highlights the potential of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach for suitable relative humidity reconstructions. Finally, the reconstructed source water isotope composition ($\delta^2\text{H}_s$ and $\delta^{18}\text{O}_s$) as calculated from the coupled approach matches the source water in the climate chamber experiment ($\delta^2\text{H}_{\text{tank-water}}$ and $\delta^{18}\text{O}_{\text{tank-water}}$).

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65 1 Introduction

66 Leaf-wax-derived biomarkers, such as long chain n -alkanes, and their stable hydrogen isotopic
67 composition ($\delta^2\text{H}_{n\text{-alkane}}$) are widely applied in paleoclimatology research. Sedimentary $\delta^2\text{H}_{n\text{-alkane}}$ values
68 correlate with $\delta^2\text{H}$ of precipitation (Huang et al., 2004; Mügler et al., 2008; Sachse et al., 2004; Sauer
69 et al., 2001), confirming the high potential of $\delta^2\text{H}_{n\text{-alkane}}$ to establish $\delta^2\text{H}$ records of past precipitation
70 (Hou et al., 2008; Rao et al., 2009; Sachse et al., 2012). However, the alteration of the isotopic signal
71 as a result of the often unknown amount of leaf water enrichment caused by evapotranspiration can
72 be several tens of per mil. This poses a challenge for accurate data interpretation (e.g. Zech et al.,
73 2015), especially in respect of single proxy ($\delta^2\text{H}_{n\text{-alkane}}$)-based climate records. Apart from studies of
74 sedimentary cellulose (Heyng et al., 2014; Wissel et al., 2008), the oxygen stable isotope composition
75 of sugar biomarkers ($\delta^{18}\text{O}_{\text{sugar}}$) emerged as complementary paleoclimate proxy during the last decade
76 (Hepp et al., 2015, 2017, Zech et al., 2013a, 2014a). The interpretation of the $\delta^{18}\text{O}_{\text{sugar}}$ values is
77 comparable to those of $\delta^2\text{H}_{n\text{-alkane}}$. When sugars originate primarily from leaf biomass of higher
78 terrestrial plants, they reflect the plant source water (which is often directly linked to the local
79 precipitation) modified by evapotranspirative enrichment of the leaf water (Tuthorn et al., 2014; Zech
80 et al., 2014a). The coupling of $\delta^2\text{H}_{n\text{-alkane}}$ with $\delta^{18}\text{O}_{\text{sugar}}$ values allows quantification of leaf-water isotopic
81 enrichment and relative air humidity (Zech et al., 2013a). This approach was validated by Tuthorn et
82 al. (2015) by applying it to topsoil samples along a climate transect in Argentina. Accordingly, the
83 biomarker-derived relative air humidity values correlate significantly with actual air relative humidity
84 from the respective study sites, highlighting the potential of the $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer
85 approach.

86 The coupled approach is based on the observation that the isotope signature of precipitation
87 ($\delta^2\text{H}_{\text{precipitation}}$ and $\delta^{18}\text{O}_{\text{precipitation}}$) typically plots on or adjacent to the global meteoric water line (GMWL),
88 in a $\delta^2\text{H}\text{-}\delta^{18}\text{O}$ diagram. The GMWL is characterized by the equation $\delta^2\text{H}_{\text{precipitation}} = 8 \cdot \delta^{18}\text{O}_{\text{precipitation}} + 10$
89 (Dansgaard, 1964). In most cases, the local precipitation can be directly linked to the source water of
90 plants, which is indeed soil water and eventually shallow groundwater. The isotopic composition of
91 xylem water of plants readily reflects these sources (e.g. Dawson, 1993). However, leaf-derived
92 biomarkers reflect the leaf water isotope composition, which is, unlike xylem water, prone to
93 evapotranspiration (e.g. Barbour and Farquhar, 2000; Helliker and Ehleringer, 2002; Cernusak et al.,
94 2003; Barbour et al., 2004; Cernusak et al., 2005; Feakins and Sessions, 2010; Kahmen et al., 2011;
95 Sachse et al., 2012; Kahmen, Schefuß, et al., 2013; Tipple et al., 2013; Lehmann et al., 2017; Liu et al.,
96 2017). During daytime, the leaf water is typically enriched in the heavy isotope compared to the source
97 water because of the evapotranspirative enrichment through the stomata. Thereby, lighter water
98 isotopes evaporate preferentially, which results in a deuterium-excess in the remaining water
99 compared to the precipitation water ($d = \delta^2\text{H} - 8 \cdot \delta^{18}\text{O}$; according to Dansgaard, 1964). The degree of
100 evapotranspirative enrichment is mainly controlled by the relative air humidity in the direct
101 surrounding of the plant leaves (e.g. Cernusak et al., 2016). Although the biomarkers reflect the
102 isotopic composition of leaf water, there is still a modification by the so-called biosynthetic
103 fractionation during the biosynthesis, leading to an offset between leaf water and biomarker isotope
104 composition. In case the biosynthetic fractionation is known and constant, there is a great potential
105 that relative humidity can be derived from coupling $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ values.

106 The overall aim of this study is to evaluate the $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach by
107 applying it to plant leaf material from three different plants grown in a climate chamber experiment
108 under well controlled conditions. More specifically, we address the following questions:

- 109 (i) which homologue and specific monosaccharide can be used to gain $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$
110 results for the climate chamber plants leaf material, respectively,

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- 111 (ii) how precisely do $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ values allow reconstructing $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of leaf
- 112 water, respectively,
- 113 (iii) how accurately does the leaf-water-isotope composition reflect the relative humidity
- 114 conditions,
- 115 (iv) and does the coupling of $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ enable a robust source water calculation
- 116 and how reliable are relative humidity reconstructions?
- 117

118 2 Material and Methods

119 2.1 Climate chamber experiment

120 A phytotron experiment was conducted at the Helmholtz Zentrum München in Neuherberg during
 121 winter 2000/2001 (Mayr, 2002). Three different dicotyledon plant species (*Eucalyptus globulus*, *Vicia*
 122 *faba* var. *minor* and *Brassica oleracea* var. *medullosa*) were grown in eight chambers for 56 days under
 123 seven distinct climatic conditions (same conditions in chambers 4 and 8). Air temperature (T_{air}) were
 124 set to 14, 18, 24 and 30°C and relative humidity (RH_{air}) to around 20, 30, 50, and 70% between 11
 125 a.m. and 4 p.m. (Fig. 1B). During the rest of the day typical natural diurnal variations were aimed for
 126 (details in Mayr, 2002). Furthermore, uniform irrigation conditions were guaranteed via an automatic
 127 irrigation system, which was controlled by tensiometers installed in 9 cm substrate depth. The tank
 128 water used for irrigation was sampled periodically (intervals of one to three days) over the whole
 129 experiment and revealed only minor variability in its isotope composition ($\delta^{18}\text{O}_{\text{tank-water}} = -10.7 \pm 0.3\text{‰}$
 130 standard deviation (σ); $\delta^2\text{H}_{\text{tank-water}} = -7 \pm 1\text{‰}$ σ). Once a week, soil water (via ceramic cups in 13 cm soil
 131 depth) and atmospheric water vapor (via dry ice condensation traps) was sampled ($\delta^2\text{H}_{\text{soil-water}}$, $\delta^{18}\text{O}_{\text{soil-}}$
 132 water and $\delta^2\text{H}_{\text{atmospheric-water-vapor}}$, $\delta^{18}\text{O}_{\text{atmospheric-water-vapor}}$). Additionally, leaf temperatures (T_{leaf}) were
 133 derived from gas exchange measurements, at least once a week (Mayr, 2002).

134 In order to analyze stable hydrogen and oxygen isotopic composition of leaf ($\delta^2\text{H}_{\text{leaf-water}}$, $\delta^{18}\text{O}_{\text{leaf-water}}$)
 135 and stem water, the plants were harvested at the end of the experiment. The vacuum distillation
 136 method was used for the extraction of the plant water. It should be noted that stem water is a mixture
 137 between phloem and xylem water, while the latter should reflect the isotopic composition of the soil
 138 water. For simplification, stem water is referred to as xylem water in the following ($\delta^2\text{H}_{\text{xylem-water}}$,
 139 $\delta^{18}\text{O}_{\text{xylem-water}}$).

140 For more details about the experiment, the reader is referred to the original publication (Mayr, 2002).

142 2.2 Leaf biomarker extraction and compound-specific stable isotope analysis

143 A total of 24 leaf samples were prepared according to Schäfer et al. (2016) for compound specific $\delta^2\text{H}$
 144 measurements of *n*-alkanes, at the Institute of Geography, Group of Biogeochemistry and
 145 Paleoclimate, University of Bern. Microwave extraction with 15 ml dichloromethane (DCM)/methanol
 146 (MeOH) 9:1 (v:v) at 100°C for 1 h was conducted. The resulting total lipid extract was purified and
 147 separated using aminopropyl-silica-gel (Supelco, 45 μm) pipette columns. The hydrocarbon fraction
 148 (containing *n*-alkanes) was eluted with *n*-hexane and cleaned via silver nitrate-coated silica gel pipettes
 149 (Supelco, 60-200 mesh) and zeolite (Geokleen Ltd.) columns. The $\delta^2\text{H}$ measurements of the highest
 150 concentrated *n*-alkanes (*n*-C₂₉ and *n*-C₃₁) were performed on a GC-²H-pyrolysis-IRMS system, equipped
 151 with an Agilent 7890A gas chromatograph (GC) and IsoPrime 100 isotope-ratio-mass spectrometer
 152 (IRMS) coupled with a GC5 pyrolysis/combustion interface operating in pyrolysis modus with a Cr
 153 (ChromeHD) reactor at 1000°C. The compound-specific $\delta^2\text{H}$ values were calibrated against a standard
 154 alkane mix (*n*-C₂₇, *n*-C₂₉, *n*-C₃₃) with known isotope composition (A. Schimmelmann, University of
 155 Indiana), measured twice every six sample injections. Standard deviation of the triplicate

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measurements were typically $\leq 5\%$. The H^{3+} factor stayed constant during the course of the measurements.

Additionally, the leaf samples were dried and finely ground in preparation for $\delta^{18}O$ analysis of hemicellulose-derived sugars (modified from Zech and Glaser, 2009) at the Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin-Luther-University Halle-Wittenberg. The hemicellulose sugars were hydrolytically extracted for 4 h at $105^{\circ}C$ using 4M trifluoroacetic acid (Amelung et al., 1996) and purified via XAD-7 and Dowex 50WX8 columns. Prior to the methylboronic-acid (MBA) derivatization (4 mg of MBA in 400 μ l dry pyridine for 1 h at $60^{\circ}C$), the cleaned sugars were frozen and freeze-dried overnight (Knapp, 1979). Compound-specific $\delta^{18}O$ measurements were performed on a Trace GC 2000 coupled to a Delta V Advantage IRMS via an ^{18}O -pyrolysis reactor (GC IsoLink) and a ConFlo IV interface (all devices from Thermo Fisher Scientific, Bremen, Germany). The sample batches were measured along with embedded co-derivatized standard batches, which contained arabinose, fucose, xylose, and rhamnose in different concentrations of known $\delta^{18}O$ value. The $\delta^{18}O$ values of the standard sugars were determined via temperature conversion/elemental analysis-IRMS coupling at the Institute of Plant Sciences, ETH Zurich, Switzerland (Zech and Glaser, 2009). This procedure allows corrections for possible amount dependencies (Zech and Glaser, 2009) and ensures the "Principle of Identical Treatment" (Werner and Brand, 2001). Standard deviations for the triplicate measurements were 0.9‰ and 2.2‰ (average over all investigated samples) for arabinose and xylose, respectively. We focus on arabinose and xylose in this study because they were (i) the dominant peaks in all chromatograms, and (ii) previously found to strongly predominate over fucose (and rhamnose) in terrestrial plants, soils (Hepp et al., 2016).

All δ values are expressed in per mil as isotope ratios ($R = ^{18}O/^{16}O$ or $^2H/^1H$) relative to the Vienna Standard Mean Ocean Water (VSMOW) standard in the common delta notation ($\delta = R_{\text{sample}} - R_{\text{standard}} / R_{\text{standard}}$; e.g. Coplen, 2011).

2.3 Framework for coupling $\delta^2H_{n\text{-alkane}}$ with $\delta^{18}O_{\text{sugar}}$ results

2.3.1 Deuterium-excess of leaf water and relative humidity

The coupled approach is based on the observation that isotope composition of global precipitation plots typically close to the GMWL ($\delta^2H_{\text{precipitation}} = 8 \cdot \delta^{18}O_{\text{precipitation}} + 10$; Dansgaard, 1964; Fig. 2). The soil water and shallow groundwater, which acts as source water for plants, can often directly be related to the local precipitation. However, especially during daytime leaf water is typically enriched compared to the precipitation due to evapotranspiration through the stomata, therefore plotting right of the GMWL (Fig. 2; e.g. Allison et al., 1985; Bariac et al., 1994; Walker and Brunel, 1990). The leaf water reservoir at the evaporative sites is frequently assumed to be in isotope steady-state (Allison et al., 1985; Bariac et al., 1994; Gat et al., 2007; Walker and Brunel, 1990), meaning that the isotope composition of the transpired water vapor is in isotopic equilibrium with the source water utilized by the plants during the transpiration process. The Craig-Gordon model (e.g. Flanagan et al., 1991; Roden and Ehleringer, 1999) approximates the isotope processes in leaf water in δ terms (e.g. Barbour et al., 2004):

$$\delta_e \approx \delta_s + \epsilon^* + \epsilon_k + (\delta_a - \delta_s - \epsilon_k) \frac{e_a}{e_i}, \quad (\text{Equation 1})$$

where δ_e , δ_s and δ_a are the hydrogen and oxygen isotopic compositions of leaf water at the evaporative sites, source water and atmospheric water vapor, respectively. The equilibrium enrichment (ϵ^*) is expressed as $(1 - 1/\alpha_{LV}) \cdot 10^3$, where α_{LV} is the equilibrium fractionation between liquid and vapor in

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per mil. The kinetic fractionation parameter (ϵ_k) describes the water vapor diffusion from intracellular air space through the stomata and the boundary layer into to the atmosphere, and e_a/e_i is the ratio of the atmospheric to intracellular vapor pressure.

In a $\delta^2\text{H}$ - $\delta^{18}\text{O}$ diagram, the isotope composition of the leaf water as well as the source water can be described as deuterium-excess (d) values by using the equation of Dansgaard (1964), with $d = \delta^2\text{H} - 8 \cdot \delta^{18}\text{O}$. This allows rewriting the Eq. 1, in which hydrogen and oxygen isotopes have to be handled in separate equations, in one equation:

$$d_e \approx d_s + (\epsilon_2^* - 8 \cdot \epsilon_{18}^*) + (C_k^2 - 8 \cdot C_k^{18}) + [d_a - d_s - (C_k^2 - 8 \cdot C_k^{18})] \cdot \frac{e_a}{e_i}, \quad (\text{Equation 2})$$

where d_e , d_s and d_a are the deuterium excess values of leaf water at the evaporative sites, source water and atmospheric water vapor, respectively. The kinetic fractionation parameter (ϵ_k) is typically related to stomatal and boundary layer resistances to water flux (Farquhar et al., 1989). We used the kinetic enrichment factor (C_k) instead of ϵ_k to be close to paleo studies where direct measurements of such a plant physiological parameter are not available. The kinetic enrichment factor is derived from a more generalized form of the Craig-Gordon model for describing the kinetic isotope enrichment for ^2H and ^{18}O (C_k^2 and C_k^{18} , respectively) (Craig and Gordon, 1965; Gat and Bowser, 1991). If the plant source water and the local atmospheric water vapor are in isotope equilibrium, the term $d_a - d_s$ in Eq. 1 can be approximated by $-\epsilon^*$. Thus, Eq. 2 can be reduced to:

$$d_e \approx d_s + (\epsilon_2^* - 8 \cdot \epsilon_{18}^*) + C_k^2 - 8 \cdot C_k^{18} \cdot \left(1 - \frac{e_a}{e_i}\right). \quad (\text{Equation 3})$$

The actual atmospheric vapor pressure (e_a) and the leaf vapor pressure (e_i) in kPa can be derived from Eqs. 4 and 5 by using T_{air} and T_{leaf} , respectively:

$$e_a = 0.61365 \cdot e^{[17.502 \cdot T_{\text{air}} / (T_{\text{air}} + 240.97)]} \cdot RH_{\text{air}} \quad (\text{Equation 4})$$

$$e_i = 0.61365 \cdot e^{[17.502 \cdot T_{\text{leaf}} / (T_{\text{leaf}} + 240.97)]}, \quad (\text{Equation 5})$$

where e_a/e_i is the relative humidity calculated with the saturation vapor pressure when the leaf temperature is used in the denominator rather than the air temperature (Eq. 5), ranging between 0 and 1. In order to increase the comparability to RH_{air} , the e_a/e_i ratio calculated with T_{leaf} in Eq. 5 can be converted into RH_{leaf} by multiplication with 100. When T_{air} is used in Eq. 5, e_a/e_i represents RH_{air} (also ranging between 0 and 1, representing 0 to 100% relative humidity when multiplying with 100). It should be noted that the differences between measured RH_{leaf} and T_{leaf} with the respective air parameters (RH , T_{air}) are not very pronounced in most cases (Mayr, 2002; Kahmen et al., 2011b), revealing rather the same trends and magnitude (Fig. 1B).

With Eqs. 2 and 3, two equations are given to derive relative humidity values by rearranging them, resulting in RH_{air} and RH_{leaf} , respectively, by using either T_{air} or T_{leaf} for ϵ^* (Eqs. 6 and 7):

$$RH_{\text{leaf/air}} \approx \frac{d_e - d_s - (\epsilon_2^* - 8 \cdot \epsilon_{18}^*) - (C_k^2 - 8 \cdot C_k^{18})}{d_a - d_s - (C_k^2 - 8 \cdot C_k^{18})}, \quad (\text{Equation 6})$$

$$RH_{\text{leaf/air}} \approx 1 - \frac{d_e - d_s}{(\epsilon_2^* - 8 \cdot \epsilon_{18}^*) + C_k^2 - 8 \cdot C_k^{18}}. \quad (\text{Equation 7})$$

Equilibrium fractionation parameters (ϵ_2^* and ϵ_{18}^*) are derived from empirical equations of Horita and Wesolowski (1994) by using either the climate chamber T_{air} or T_{leaf} values. The kinetic fractionation parameters (C_k^2 and C_k^{18}) for ^2H and ^{18}O , respectively, are set to 25.1 and 28.5‰ according to Merlivat (1978), who reported maximum values during the molecular diffusion process of water through a stagnant boundary layer. It should be noted that ϵ_k values of broadleaf trees and shrubs over broad

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climatic conditions are well in the range with used C_k^2 and C_k^{18} values, revealing 23.9 ± 0.9 and 26.7%
 ± 1.0 for ϵ_k^2 and ϵ_k^{18} , respectively (derived from supplementary data of Cernusak et al., 2016).
 If $\delta^2H_{\text{leaf-water}}$ and $\delta^{18}O_{\text{leaf-water}}$ can be reconstructed from the measured δ values of n -alkanes and sugars
 biomarkers, this framework provides a powerful tool to establish relative humidity records from
 sedimentary archives (Hepp et al., 2017; Zech et al., 2013a). To reconstruct the isotope composition of
 leaf water it is assumed that fractionation factors of -160% for 2H of alkanes $n\text{-C}_{29}$ and $n\text{-C}_{31}$ (ϵ_{bio}^2 ;
 Sachse et al., 2012; Sessions et al., 1999), and $+27\%$ for ^{18}O of the hemicellulose-derived sugars
 arabinose and xylose ($\epsilon_{\text{bio}}^{18}$; Cernusak et al., 2003; Schmidt et al., 2001; Sternberg et al., 1986; Yakir
 and DeNiro, 1990) can be applied:

$$\text{alkane-based } \delta^2H_{\text{leaf-water}} = (\delta^2H_{n\text{-alkane}} - \epsilon_{\text{bio}}^2) / (1 + \epsilon_{\text{bio}}^2/1000) \quad (\text{Equation 8})$$

$$\text{sugar-based } \delta^{18}O_{\text{leaf-water}} = (\delta^{18}O_{\text{sugar}} - \epsilon_{\text{bio}}^{18}) / (1 + \epsilon_{\text{bio}}^{18}/1000). \quad (\text{Equation 9})$$

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2.3.2 Isotope composition of plant source water

In a δ^2H - $\delta^{18}O$ diagram, the hydrogen and oxygen isotope composition of the plant source water (δ^2H_s
 and $\delta^{18}O_s$, respectively) can be assessed via the slope of the individual leaf water evapotranspiration
 lines (LEL's; Craig and Gordon, 1965; Gat and Bowser, 1991). Depending on the degree of
 simplification, the LEL slope (S_{LEL}) can be derived from Eq. 10 (consistent to Eq. 2) and Eq. 11 (consistent
 to Eq. 3):

$$S_{\text{LEL}} \approx \frac{\epsilon_2^* + C_k^2 + (\delta_a^2 - \delta_s^2 - C_k^2) \cdot \frac{e_a}{e_l}}{\epsilon_{18}^* + C_k^{18} + (\delta_a^{18} - \delta_s^{18} - C_k^{18}) \cdot \frac{e_a}{e_l}}, \quad (\text{Equation 10})$$

$$S_{\text{LEL}} \approx \frac{\epsilon_2^* + C_k^2 \cdot \left(1 - \frac{e_a}{e_l}\right)}{\epsilon_{18}^* + C_k^{18} \cdot \left(1 - \frac{e_a}{e_l}\right)} \approx \frac{\epsilon_2^* + C_k^2}{\epsilon_{18}^* + C_k^{18}}, \quad (\text{Equation 11})$$

where all parameters are defined as in section 2.3.1. The δ^2H_s and $\delta^{18}O_s$ values can then be calculated
 for each leaf water data point via the intersect between the individual LEL's with the GMWL. The model
 results (from Eqs. 10 and 11) can be furthermore compared to the slope calculated by Eq. 12, using the
 measured $\delta^2H_{\text{leaf-water}}$, $\delta^{18}O_{\text{leaf-water}}$ and $\delta^2H_{\text{tank-water}}$, $\delta^{18}O_{\text{tank-water}}$ values (Craig and Gordon, 1965; Gat and
 Bowser, 1991).

$$S_{\text{LEL}} = \frac{\delta^2H_{\text{leaf-water}} - \delta^2H_{\text{tank-water}}}{\delta^{18}O_{\text{leaf-water}} - \delta^{18}O_{\text{tank-water}}} \quad (\text{Equation 12})$$

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2.4 Modeling and isotope fractionation calculations

Relative humidity (Eq. 6), deuterium-excess values of leaf water (d_e , Eq. 2) and S_{LEL} values (Eq. 10) were
 modeled leading to less simplified results, because the measured δ_a values are used explicitly.
 Equations 7, 3 and 11 were therefore used to obtain RH, d_e and S_{LEL} results, representing a more
 simplified model approach because $\delta_a - \delta_s$ are approximated by $-\epsilon^*$. This model procedure allows
 furthermore the comparison of scenarios based on air or leaf temperature (T_{air} or T_{leaf}). In Eqs. 6 and
 7, the reconstructed (biomarker-based) deuterium-excess $S_{\text{leaf-water}}$ was used as additional input, as
 gained from Eqs. 8 and 9. The modeled LEL slopes (Eqs. 10 and 11) were used to derive source water
 isotope composition (δ^2H_s , $\delta^{18}O_s$). In all equations presented in section 2.3 to gain the model results
 (Eqs. 2 to 8), $\delta^2H_{\text{atmospheric-water-vapour}}$, $\delta^{18}O_{\text{atmospheric-water-vapour}}$ and $\delta^2H_{\text{tank-water}}$, $\delta^{18}O_{\text{tank-water}}$ were used for δ_a
 and δ_s (therefore also for d_a and d_s). All other input parameters were set as described in section 2.3. In
 order to provide an 1 σ range bracketing the modeled results (d_e , RH_{air} , RH_{leaf} , S_{LEL} , δ^2H_s , $\delta^{18}O_s$), the

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calculations were also run with values generated by subtracting/adding the individual σ to the average. This procedure was also used to derive measured deuterium-excess_{leaf-water} and S_{LEL} uncertainties. Model quality was overall assessed by calculating the coefficient of determination [$R^2 = 1 - \sum (\text{modeled} - \text{measured})^2 / \sum (\text{measured} - \text{measured mean})^2$] and the root mean square error $\left[\text{RMSE} = \sqrt{\left(\frac{1}{n} \cdot \sum (\text{modeled} - \text{measured})^2 \right)} \right]$. The R^2 is not equal to the r^2 , which provides here the fraction of variance explained by a linear regression between a dependent (y) and an explanatory variable [$r^2 = 1 - \sum (y - \text{fitted } y)^2 / \sum (y - \text{mean } y)^2$] (R Core Team, 2015).

The fractionation between the measured leaf biomarkers and leaf water can be described by the following equations (Eq. 10 and 11; e.g. Coplen, 2011):

$$\epsilon_{n\text{-alkane/leaf-water}} = (\delta^2\text{H}_{n\text{-alkane}} - \delta^2\text{H}_{\text{leaf-water}}) / (1 + \delta^2\text{H}_{\text{leaf-water}}/1000) \quad (\text{Equation 13})$$

$$\epsilon_{\text{sugar/leaf-water}} = (\delta^{18}\text{O}_{\text{sugar}} + \delta^{18}\text{O}_{\text{leaf-water}}) / (1 + \delta^{18}\text{O}_{\text{leaf-water}}/1000). \quad (\text{Equation 14})$$

For Eqs. 8 and 9 (biomarker-based leaf water reconstruction) as well as for Eqs. 13 and 14, the 1σ range were calculated by subtracting/adding the individual σ , analogous to the modeling results.

All calculations and statistical analysis were realized in R (version 3.2.2; R Core Team, 2015).

3 Results and Discussion

3.1 Compound-specific isotope results of leaf wax-derived n -alkanes and hemicellulose-derived sugars

All investigated leaf material showed a dominance of C_{29} n -alkanes. The dominance of n - C_{29} in *Brassica oleracea* and *Eucalyptus globulus* was also reported by Ali et al. (2005) and Herbin and Robins (1968). *Vicia faba* leaf samples additionally revealed a high abundance of C_{31} n -alkanes. This agrees with results from Maffei (1996) and enables a robust determination of compound-specific $\delta^2\text{H}$ values for C_{29} and C_{31} . The $\delta^2\text{H}_{n\text{-alkane}}$ values of *Vicia faba* are therefore calculated as weighted mean.

The top of Fig. 1A illustrates the $\delta^2\text{H}_{n\text{-alkane}}$ results along with isotopic data for leaf, xylem and soil water (the latter were originally published in Mayr 2002). In addition the climate chamber conditions (RH_{air} , RH_{leaf} , T_{air} and T_{leaf}) are displayed (all from Mayr, 2002; Fig. 1B). For more details about the (plant) water isotope results, climate chamber conditions as well as not shown plant physiological properties the reader is referred to Mayr (2002). The $\delta^2\text{H}_{n\text{-alkane}}$ values range from -213 to -144‰ over all plant species. As revealed by overlapping notches in the respective boxplots, no statistically significant differences in the median values between the three plant species can be described (Fig. S1A; McGill et al., 1978). Fig. 1A moreover shows that $\delta^2\text{H}_{n\text{-alkane}}$ values range largest for *Eucalyptus globulus* compared to the other two plants. However, the low number of samples per plant species prohibits a robust interpretation.

(Fig. 1)

The investigated leaf samples yielded substantially higher amounts of arabinose and xylose compared to fucose and rhamnose. This is in agreement with sugar patterns reported for higher plants (D'Souza et al., 2005; Hepp et al., 2016; Jia et al., 2008; Prietzel et al., 2013; Zech et al., 2012, 2014a) and hampers a robust data evaluation of fucose and rhamnose. The $\delta^{18}\text{O}$ values of the investigated pentoses arabinose and xylose range from 30 to 47‰ and 30 to 50‰, respectively, and are shown

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along with isotopic data for leaf, xylem and soil water (Mayr 2002) in the bottom of Fig. 1A. No considerable difference in the $\delta^{18}\text{O}$ values of arabinose and xylose can be seen in the $\delta^{18}\text{O}$ pentose data. This is in line with findings from Zech and Glaser (2009), Zech et al. (2012), Zech et al. (2013b) and Zech et al. (2014b) but contradicting with slightly more positive $\delta^{18}\text{O}_{\text{arabinose}}$ values compared to $\delta^{18}\text{O}_{\text{xylose}}$ values reported by Zech et al. (2013a) and Tuthorn et al. (2014). Overall, the two sugars display very similar results (Fig. 1; $r^2 = 0.7$, $p < 0.001$, $n = 24$). The $\delta^{18}\text{O}$ values of arabinose and xylose can therefore be combined as a weighted mean (as $\delta^{18}\text{O}_{\text{sugar}}$ values) for further data interpretation. The $\delta^{18}\text{O}_{\text{sugar}}$ values are not significantly different between the three investigated plant species.

The compound-specific isotope results of leaf hemicellulose-derived sugars and leaf wax-derived n -alkanes can be compared with leaf, xylem, soil and tank water (compare Fig. 1A and Fig. 2). This comparison reveals that soil and xylem water plot close to the tank water, whereas leaf water shows a clear evapotranspirative enrichment. This enrichment strongly differs between the climate chambers, depending mainly on T and RH conditions. The biomarker results furthermore follow the leaf water with a certain offset (ϵ_{bio}).

(Fig. 2)

3.2 Do n -alkane and sugar biomarkers reflect the isotope composition of leaf water?

The $\delta^2\text{H}_{n\text{-alkane}}$ dataset reveals a significant correlation with $\delta^2\text{H}_{\text{leaf-water}}$ of 0.45 (r^2) using all plant species with $p < 0.001$ (Fig. 3A). A slope of 1.1 and an intercept of -152‰ furthermore characterize the relationship. It seems that each plant type shows a different $\delta^2\text{H}_{n\text{-alkane}}$ to $\delta^2\text{H}_{\text{leaf-water}}$ relation, with the highest slope for *Vicia faba* and the lowest for *Brassica oleracea*. However, we argue that the number of replicates for each plant species is simply too low to interpret this finding robustly. A highly significant correlation is also observed for the correlation between $\delta^{18}\text{O}_{\text{sugar}}$ and $\delta^{18}\text{O}_{\text{leaf-water}}$ ($r^2 = 0.84$, $p < 0.001$; Fig. 3B). The regression reveals a slope of 0.74 and an intercept of 30.7‰ .

(Fig. 3)

Since it is well known that measured leaf water is not always equal to the specific water pool in which the n -alkanes are biosynthesized (e.g. Tipple et al., 2015), the correlation reveals a rather low r^2 (Fig. 3A). Furthermore, NADPH is acting also as hydrogen source during n -alkane biosynthesis, which is clearly more negative than the biosynthetic water pool (Schmidt et al., 2003), further contributing to a weakening of the $\delta^2\text{H}_{n\text{-alkane}}$ to $\delta^2\text{H}_{\text{leaf-water}}$ relationship. The correlation between the deuterium contents of leaf wax n -alkanes and leaf water presented here is still well in range with the literature. Feakins and Sessions (2010) presented n -alkane (C_{29} and C_{31}) and leaf water $\delta^2\text{H}$ data from typical plant species (excluding grasses) along a southern California aridity gradient, revealing that only $\delta^2\text{H}$ of $n\text{-C}_{29}$ is significantly correlated with leaf water ($r^2 = 0.24$, $p < 0.1$, $n = 16$; based on the associated supplementary data). Another field dataset from the temperate forest at Brown's Lake Bog, Ohio, USA revealed significant correlations between $\delta^2\text{H}$ of $n\text{-C}_{29}$ and $n\text{-C}_{31}$ with leaf water of the species *Prunus serotina*, *Acer saccharinum*, *Quercus rubra*, *Quercus alba*, and *Ulmus americana* ($r^2 = 0.49$, $p < 0.001$, $n = 38$; $r^2 = 0.59$, $p < 0.001$, $n = 29$; as derived from the supplement material of Freimuth et al., 2017). Data from a controlled climate chamber experiment using two tree species show a highly significant relationship between leaf wax n -alkanes $\delta^2\text{H}$ and leaf water (with C_{31} of *Betula occidentalis* and C_{29} of *Populus fremontii*; $r^2 = 0.96$, $p < 0.001$, $n = 24$; derived from supplementary data of Tipple et al., 2015). It is conformed that leaf wax n -alkanes of dicotyledonous plants largely incorporate the leaf water

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isotope signal, while in monocotyledonous plants (e.g. grasses) the *n*-alkanes are more strongly affected by the source water due to the leaf growth at the intercalary meristem (Kahmen et al., 2013). The observed slope of the $\delta^{18}\text{O}_{\text{sugar}}$ to $\delta^{18}\text{O}_{\text{leaf-water}}$ relationship (Fig. 3B) could serve as indicator for a leaf water (enrichment) signal transfer damping of approximately 26%. The theory behind the signal damping is adopted from the cellulose research (e.g. Barbour and Farquhar, 2000). Barbour and Farquhar (2000) related the extent of the signal damping to the proportion of unenriched source water, which contribute to the local synthesis water pool and to the proportion of exchangeable oxygen during cellulose synthesis. Here calculated damping factor would be well in the range of values reported for cellulose synthesis in *Gossypium hirsutum* leaves (between 35 and 38%; Barbour and Farquhar, 2000), for *Eucalyptus globulus* leaf samples (38%; Cernusak et al., 2005) and for five C_3 and C_4 grasses (25%; Helliker and Ehleringer, 2002). Recently Cheesman and Cernusak (2017) provided damping factors for leaf cellulose synthesis based on plant data grown under same conditions at Jerusalem Botanical Gardens published by Wang et al. (1998), ranging between 4 and 100% with a mean of 49%, revealing large variations among and between ecological groups (namely conifers, deciduous, evergreen and shrubs). A large range of damping factors associated with leaf cellulose was also reported by Song et al. (2014) for *Ricinus communis* grown under controlled conditions. A common disadvantage of the above-mentioned studies is the absence of direct measurements of the proportion of depleted source water contribution to the local synthesis water (as noticed by Liu et al., 2017), which largely contribute to the extent of the damping factor (Barbour and Farquhar, 2000). However, when transferring cellulose results to pentoses, such as hemicellulose-derived arabinose and xylose, it should be noted that they are biosynthesized via decarboxylation of the carbon at position six (C6) from glucose (Altermatt and Neish, 1956; Burget et al., 2003; Harper and Bar-Peled, 2002). Waterhouse et al. (2013) showed that the oxygen atoms at C6 position in glucose moieties, used for heterotrophic cellulose synthesis, are strongly affected by the exchange with local water (up to 80%). Based on these findings, it can be suggested that the influence of the non-enriched source water during the synthesis of leaf hemicelluloses is rather small.

3.3 Fractionation factors between biomarkers and leaf water

In order to explore possible species-specific effects on the fractionation between the biomarkers and the leaf water, boxplots of the individual plant species of $\epsilon_{n\text{-alkane/leaf-water}}$ and $\epsilon_{\text{sugar/leaf-water}}$ values are shown in Fig. 4. Median $\epsilon_{n\text{-alkane/leaf-water}}$ values are -155‰ for *Brassica oleracea*, -164‰ for *Eucalyptus globulus* and -149‰ for *Vicia faba* (Fig. 4A), with an overall mean value of -156‰ (ranging from -133 to -192‰). Median $\epsilon_{\text{sugar/leaf-water}}$ values of +27.0‰ for *Brassica oleracea*, +26.6‰ for *Eucalyptus globulus*, +26.8‰ for *Vicia faba* are shown in Fig. 4B. The overall $\epsilon_{\text{sugar/leaf-water}}$ average value of the three investigated species is +27.3‰ (ranging from +23.0 to +32.3‰). In both plots, no difference between the individual species seems to be observable.

(Fig. 4)

The boxplots of $\epsilon_{n\text{-alkane/leaf-water}}$ reveal that the median of the three investigated plant species can be statistically not distinguished, due to overlapping notches (Fig. 4A). It should be noted that due to the low sample number from each species, the 95% confidence interval is larger than the interquartile range in some cases. However, it seems that at least small species-specific differences cannot be ruled out. Our $\epsilon_{n\text{-alkane/leaf-water}}$ values resemble well the data from a laboratory study (Kahmen et al., 2011), reporting a median value of -162‰ for *n*- C_{25} , *n*- C_{27} and *n*- C_{29} of *Populus trichocarpa*. Furthermore, they are well comparable to climate chamber data of *Betula occidentalis* (*n*- C_{31}) and *Populus fremontii* (*n*-

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C₂₉) from Tipple et al. (2015), reporting a median $\epsilon_{n\text{-alkane/leaf-water}}$ value of -155‰. In addition, field experiments reveal similar median values of -151‰ (for $n\text{-C}_{29}$) and -142‰ (for $n\text{-C}_{31}$) from typical plant species (excluding grasses) from southern California (Feakins and Sessions, 2010) and -144‰ (for $n\text{-C}_{29}$, of the species *Prunus serotina*, *Acer saccharinum*, *Quercus rubra*, *Quercus alba* and *Ulmus americana*) from the temperate forest at Brown's Lake Bog, Ohio, USA. The large range in $\epsilon_{\text{xylem-water/leaf-water}}$ values from our study (-192 to -133‰) is also obvious in the respective laboratory and field studies (-198 to -115‰, derived from $n\text{-C}_{29}$ and $n\text{-C}_{31}$ data from Feakins and Sessions, 2010; Kahmen et al., 2011a; Tipple et al., 2015; Freimuth et al., 2017). This could point to a specific water pool being used rather than bulk leaf water during biosynthesis (Sachse et al., 2012; Schmidt et al., 2003). In more detail, alkane synthesis takes place by modifying/expanding fatty acids in the cytosol, while fatty acids are synthesized in the chloroplasts (Schmidt et al., 2003). Thus, the cytosol as well as chloroplast water is one hydrogen source. However hydrogen can additionally be added to the alkanes and fatty acids by NADPH which originates from different sources (photosynthesis and pentose phosphate cycle, Schmidt et al., 2003). It is therefore challenging to measure directly the water pool in which the alkanes are biosynthesized (Tipple et al., 2015). Moreover, biosynthetic and metabolic pathways in general (Kahmen et al., 2013; Sessions et al., 1999; Zhang et al., 2009), the carbon and energy metabolism of plants more specifically (Cormier et al., 2018) and the number of carbon atoms of the $n\text{-alkane}$ chains (Zhou et al., 2010) may have an influence on the fractionation. Our $\epsilon_{n\text{-alkane/leaf-water}}$ values correlate with T_{air} (Fig. S2A), whereas the correlation with RH_{air} (Fig. S2B) is not significant. This could point to a relationship between $\epsilon_{\text{xylem-water/leaf-water}}$ and plant physiological processes (affecting various plants differently).

The $\epsilon_{\text{sugar/leaf-water}}$ values (Fig. 4B) do not correlate significantly with T_{air} , but significantly with RH_{air} (Fig. S2C and D). A temperature dependence of the $\epsilon_{\text{sugar/leaf-water}}$ is not supported by this experiment, in contrast to results from Sternberg and Ellsworth (2011), where a temperature effect on oxygen fractionation during heterotrophic cellulose biosynthesis is observed. The here observed fractionation between hemicellulose-derived sugars and leaf water, with regard to $\epsilon_{\text{sugar/leaf-water}}$ values, is well in range with values reported for sucrose (exported from photosynthesizing leaves) and leaf water, which was shown to be +27‰ (Cernusak et al., 2003). Also the cellulose biosynthesis is associated with an enrichment of around +27‰ compared to the synthesis water as shown in growth experiments (Sternberg et al., 1986; Yakir and DeNiro, 1990). The relatively uniform fractionation is explained via the isotope exchange between the carbonyl oxygens of the organic molecules and the surrounding water (cf. Schmidt et al., 2001). This equilibrium fractionation effect was indeed described earlier by the reversible hydration reaction of acetone in water by Sternberg and DeNiro (1983) to be +28, +28 and +26‰ at 15, 25 and 35°C, respectively. However, the observed range of approximately 9‰ (Fig. 4B) could indicate that partially more than the oxygen equilibrium fractionation between organic molecules and medium water have to be considered. Presumably, isotopic as well as sucrose synthesis gradients within the leaf have to be taken into account when interpreting leaf sugar oxygen isotopic compositions and their correlation to leaf water (Lehmann et al., 2017). Lehmann et al. (2017) reported on a fractionation between sucrose and leaf water of +33.1‰. Based on this they proposed a conceptual scheme how such gradients can lead to discrepancies between the isotopic composition of the bulk leaf water and the synthesis water, while the latter is incorporated into the carbohydrates, and thus fractionation determination based on bulk leaf water can exceed the common average of +27‰. Also Mayr et al. (2015) found a fractionation between aquatic cellulose $\delta^{18}\text{O}$ and lake water larger than this value of around +29‰.

3.4 Strong control of relative humidity over deuterium-excess of leaf water

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The correlations between leaf water-based and measured RH_{air} or RH_{leaf} as well as modeled d_e and measured deuterium-excess_{leaf-water} are illustrated in Fig. 5A, B, D and E. Furthermore, modeled LEL slopes are compared to measured LEL slopes in Fig. 5C and F. In red, the results of the less simplified models are displayed (Eqs. 6, 2 and 10), in black the results of the more simplified models are shown (Eqs. 7, 3 and 11).

(Fig. 5)

Evidence for the strong control of relative humidity on deuterium-excess of leaf water comes from multivariate regression analysis between the measured deuterium-excess_{leaf-water} values versus RH_{air} , RH_{leaf} and T_{air} , T_{leaf} . The results reveal that the deuterium-excess_{leaf-water} significantly correlates with RH_{air} of the climate chambers ($p < 0.001$), with an r^2 of 0.92. When RH_{leaf} and T_{leaf} values are used, the r^2 is 0.84 and deuterium-excess_{leaf-water} correlates significantly with RH_{leaf} ($p < 0.001$). The strong control of relative humidity on deuterium-excess of leaf water is furthermore supported by the significant correlations between calculated versus measured RH_{air} values (Fig. 5A), regardless of whether the Eq. 6 or 7 were used (representing a lower and higher degree of simplification). This is in line with the strong correlation between modeled d_e based on T_{air} and measured deuterium-excess_{leaf-water} values (Fig. 5B). When modeled RH_{leaf} values are compared to the measured ones, the correlation is less strong compared to RH_{air} (Fig. 5D vs. 5A), represented by lower R^2 and higher RMSE values. Clearly more data points are lying above the 1:1 line with regard to RH_{leaf} , compared to RH_{air} . On the same basis, the T_{leaf} -based d_e shows a weaker correlation to the measured values than the T_{air} -based d_e (Fig. 5E vs. 5B). The generally better model performance when T_{air} is used (in contrast to T_{leaf}) could point to the fact that T_{leaf} does not well represent the actual conditions in the leaves. For the correlation between modeled and measured RH_{leaf} this means that the measured RH_{leaf} values do not reflect the real conditions because measured RH_{leaf} is calculated via $e_l/e_a \cdot 100$ with T_{leaf} as input for the e_a equation (see section 2.3). In fact, the RH model results do not differ from each other and can be well compared to the measured RH_{air} , while the measured RH_{leaf} values reveal an average offset of approximately 9% with regard to the median values (Figure S3A). This can be explained by the small difference in ϵ^* calculated either with T_{leaf} or T_{air} . Moreover, when T_{leaf} values are used to model d_e , the match to T_{air} -based d_e and measured deuterium-excess_{leaf-water} values is weaker (Fig. 5B vs. E; Fig. S3B). This offset is caused by higher T_{leaf} values (compared to T_{air} ; Fig. 1), which are leading to more negative modeled d_e values.

Overall, the modeled d_e values show a high agreement with measured deuterium-excess of leaf water despite without being too positive, which can be expected from the literature. This is because bulk leaf is less enriched than the leaf water at the evaporative sites, which is however, the output of the Craig-Gordon-based leaf water enrichment model (e.g. Allison et al., 1985; Barbour et al., 2004; Cernusak et al., 2016; section 2.3). Especially under low relative humidity conditions, the discrepancy between Craig-Gordon model results and the measured values is shown to be more pronounced, associated with higher transpiration fluxes and higher isotope heterogeneity within the leaf water due to a non-uniform closure of the stomata (Flanagan et al., 1991; Santrucek et al., 2007). An overestimation of the Craig-Gordon models can hardly be observed here (Fig. 5B and 5E). However, based on the accepted leaf water enrichment theory (e.g. Cernusak et al., 2016), higher transpiration rates (e.g. under low humidity conditions) should still lead to a larger discrepancy between Craig-Gordon modelled and measured leaf water, because the back diffusion of enriched leaf water from the evaporative sites should get lower the higher the transpiration flux is. Why there is no difference between modeled and

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measured deuterium-excess of leaf water in here presented climate chamber experiment is not comprehensible. The simplified model variants show generally a better correspondence between calculated and measured deuterium-excess of leaf water, based on R^2 and RMSE, than the less simplified models. This does not seem to be related to the slope of the LEL because it can only be linked to the measured values based on the less simplified models (Fig. 5C and 5F). The simplified air and leaf temperature based slopes average at 2.7 and 2.6, respectively, with a common range between 2.5 and 2.8. The average is well in agreement with the mean measured S_{LEL} of 2.9. In addition, a regression through the tank water and all leaf water points reveals a slope of 2.7 (± 0.02 , based on subtracting/adding the individual σ ; $r^2 = 0.98$, $n = 48$, $p < 0.001$). This could be the reason why the more simplified models are still more accurate, despite the less simplified models do not reflect well the range of the measured S_{LEL} , which vary between 2.4 and 3.8. Much better matches are found for the less simplified LEL slopes (T_{air} based: 2.6 and 3.8, T_{leaf} based: 2.5 and 3.5; Fig. 5C and 5F). Indeed the measured as well as the calculated S_{LEL} depend on the e_a/e_i ratio (hence RH_{leaf} and RH_{air} regarding T_{leaf} or T_{air} is used for calculations, respectively) and on $\delta_a - \delta_s$, in line with the theory and literature (see section 2.3; e.g. Allison et al., 1985). The higher accuracy of the simpler models would therefore imply that the S_{LEL} depend only on equilibrium and kinetic fractionation parameters for both isotopes, which would valid for isotope equilibrium conditions between the tank water (the water source of the plants) and the atmospheric water vapor, allowing the usage of the unambiguous approximation $\delta_a - \delta_s = -\epsilon^*$. Indeed, close-to equilibrium conditions between the tank water and the atmospheric water vapor are observed for the climate chambers 4 to 6 and 8, while the others are characterized by a slight disequilibrium conditions. However, the degree of uncertainty seems to be higher when using d_a values, by the probably inadequate representation of the measured $\delta^2H_{atmospheric-water-vapor}$ and $\delta^{18}O_{atmospheric-water-vapor}$ with the actual conditions influencing the plants in the climate chamber, leading to a generally better performance of the more simplified model variants.

3.5 Coupling $\delta^2H_{n-alkane}$ and $\delta^{18}O_{sugar}$ – Potential and limitations

One of the advantages of the proposed coupled $\delta^2H_{n-alkane}$ - $\delta^{18}O_{sugar}$ approach is a more robust reconstruction of the isotope composition of the source water, which can often be directly linked to the local precipitation signal (Hepp et al., 2015, 2017; Tuthorn et al., 2015; Zech et al., 2013a). Therefore, Fig. 6 shows boxplots for measured leaf water, biomarker-based (reconstructed) leaf water, measured source water (tank water; see section 2.1), biomarker-based source water (using reconstructed leaf water as origin for the LEL's) and leaf-water-based source water values (using measured leaf water as origin for the LEL's). Source water isotope compositions were calculated via the slopes of the LEL's and the GMWL. The numbers (1-4) mark the available scenarios for source water reconstruction (see section 2.4): 1) S_{LEL} calculated with the more simplified Eq. 11 with T_{air} , 2) as 1 but with T_{leaf} , 3) S_{LEL} calculated with Eq. 10 with T_{air} , 4) as 3 but with T_{leaf} . Fig. 6 clearly shows that the n -alkane and sugar biomarkers reflect leaf water rather than tank water used for irrigation. For δ^2H , neither the range nor the median of the $\delta^2H_{leaf-water}$ are well captured by the alkane-based leaf water values. However, the overlapping notches do not support a statistical difference in the median values (Fig. 6A). The medians are still on average 13‰ more positive than the measured $\delta^2H_{tank-water}$. A higher agreement between measured and modeled values is observed from leaf water-based δ^2H_s compared to $\delta^2H_{tank-water}$. The average offset is reduced to 2‰ and the range is reduced by approximately 70‰, compared to the biomarker-based reconstruction. Besides the more simplified leaf water-based δ^2H_s using T_{leaf} for calculating ϵ^* (scenario 2 in Fig. 6A), no statistical significant difference can be seen between the leaf water-based δ^2H_s and the $\delta^2H_{tank-water}$, with regard to the overlapping notches.

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(Fig. 6)

For $\delta^{18}\text{O}$, the sugar-based leaf water values are in agreement with the measured ones with regard to the median values, as supported by the largely overlapping notches (Fig. 6B). The range of the reconstructed leaf water is in the order of 6‰ smaller than for the measured $\delta^{18}\text{O}_{\text{leaf-water}}$ dataset. All reconstructed $\delta^{18}\text{O}_s$ values, regardless whether they are biomarker- or leaf water-based, are comparable to the measured $\delta^{18}\text{O}_{\text{tank-water}}$. While the biomarker-based datasets depict an average offset of 2‰, the leaf water-based values only differ by 0.3‰ from the tank water $\delta^{18}\text{O}$ values, referring to the medians. As for $\delta^2\text{H}$, the same leaf water-based $\delta^{18}\text{O}_s$ scenario (more simplified leaf water-based model using T_{leaf} for calculating ϵ^* , scenario 2 in Fig. 6B) do not show overlapping notches with $\delta^{18}\text{O}_{\text{tank-water}}$, while the other leaf water-based source water reconstructions do. In addition, the range in the leaf water-based $\delta^{18}\text{O}_{\text{source-water}}$ values is considerable smaller than for the biomarker-based one (9‰ reduction). The overall larger range in modeled $\delta^2\text{H}_s$ and $\delta^{18}\text{O}_s$ compared to measured $\delta^2\text{H}_{\text{tank-water}}$ and $\delta^{18}\text{O}_{\text{tank-water}}$ can be related to uncertainties in S_{LEL} modeling (see equations in section 2.3.2). Bariac et al. (1994) mentioned that they found no agreement between the intersect of modeled LEL's with the GMWL and the plant source water. Allison et al. (1985) explained such results with changing environmental conditions, leading to various LEL's with a locus line not necessarily passing the $\delta^2\text{H}_s$ and $\delta^{18}\text{O}_s$ data point, in a system that approaches rapidly new steady-state conditions.

Finally, the alkane and sugar-based leaf water values were used to reconstruct RH_{air} and RH_{leaf} . While the measured RH_{air} is well captured by the biomarker-based air relative humidity values ($R^2 = 0.54$ and 0.48 for the more and less simplified models, respectively, Fig. 7A), the correlations are weak between the reconstructed leaf relative humidity values and the measured RH_{leaf} ($R^2 = 0.09$ and -0.04 for the more and less simplified models, respectively, Fig. 7B). The measured RH_{air} is reconstructed most accurate by the biomarker-based air relative humidity values (Fig. 7A). As for leaf water-based RH reconstructions, a difference between biomarker-based RH_{air} and RH_{leaf} is observed (compare Fig. 7B with 7A). This can be explained by the small difference between T_{leaf} and T_{air} , used for ϵ^* calculations in the respective equations. The better performance of the more simplified models compared to the less simplified ones, in general, and the fact that T_{air} seems to be the better model input compared to T_{leaf} , more specifically, can be explained as for the leaf water-based application (see section 3.3). The T_{leaf} as well as the measured $\delta^2\text{H}_{\text{atmospheric-water-vapor}}$ and $\delta^{18}\text{O}_{\text{atmospheric-water-vapor}}$ values seem to be less representative for the conditions affecting the climate chamber plant leaves.

(Fig. 7)

Overall, a lower coefficient of determination of the biomarker-based model results compared to the leaf water-based reconstructions (compare Fig. 5A and D with Fig. 7A and B) is observed. This can be attributed to the uncertainties in leaf water reconstructed using $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ datasets as discussed in section 3.2. The limitations regarding deuterium arose from the rather weak relationship between the $\delta^2\text{H}$ of the n -alkanes and the leaf water, probably linked with the large range in the fractionation between n -alkanes and leaf water ($\epsilon^2_{n\text{-alkane/leaf-water}}$). The applied equation to reconstructed $\delta^2\text{H}_{\text{leaf-water}}$ by using $\delta^2\text{H}_{n\text{-alkane}}$ and a constant biosynthetic fractionation of -160‰ (Eq. 13) was considered to be suitable (Sachse et al., 2012; Sessions et al., 1999), but introduce also some uncertainty for the final relative humidity reconstruction. With regard to oxygen, the relatively large variations in $\epsilon_{\text{sugar/leaf-water}}$ of 9‰ have to be considered (Fig. 4B), because in the $\delta^{18}\text{O}_{\text{leaf-water}}$

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reconstructions a fixed value of +27‰ is used (Eq. 14). Such a uniform biosynthetic fractionation is an approximation which may not always be fulfilled, as shown in the literature (e.g. Sternberg and Ellsworth, 2011; Lehmann et al., 2017). Especially the underestimation of the biomarker-based RH_{air} values under the 68% relative humidity conditions, as well as the large range in reconstructed RH_{air} values for the 48, 49, 50% RH_{air} chambers can be attributed to the leaf water reconstruction uncertainties. It should be mentioned that using Eqs. 8 and 9 to calculate leaf water isotope composition based on the biomarkers via a biosynthetic fractionation values implies that the fractionation process in principle can be treated as single process with a unique source. While this approximation can be questioned (see discussion in section 3.2), the overall approximation between biomarker-based and measured RH_{air} highlights the potential of the approach (Hepp et al., 2017; Tuthorn et al., 2015; Zech et al., 2013a), also for future paleo-applications.

4 Conclusions

The climate chamber results and discussion suggest that leaf wax-derived *n*-alkane and hemicellulose-derived sugar biomarkers are valuable $\delta^2H_{leaf-water}$ and $\delta^{18}O_{leaf-water}$ recorders, respectively. The coupling of $\delta^2H_{n-alkane}$ and $\delta^{18}O_{sugar}$ results allows moreover a robust RH_{air} reconstruction of the chambers in which the plants were grown, by using simplified Craig-Gordon equations. With regard to the research questions, we summarize as follows:

- (i) Alkanes with the chain-length *n*-C₂₉ were found to be suitable abundant for compound-specific δ^2H measurements in the leaf samples from all investigated species (*Eucalyptus globulus*, *Vicia faba* var. *minor* and *Brassica oleracea* var. *medullosa*). For *Vicia faba*, additionally *n*-C₃₁ could be evaluated robustly. $\delta^{18}O_{sugar}$ values could be obtained for the hemicellulose-derived monosaccharides arabinose and xylose.
- (ii) Both the $\delta^2H_{n-alkane}$ and $\delta^{18}O_{sugar}$ values yielded highly significant correlations with $\delta^2H_{leaf-water}$ and $\delta^{18}O_{leaf-water}$ ($r^2 = 0.45$ and 0.85 , respectively; $p < 0.001$, $n = 24$). Mean fractionation factors between biomarkers and leaf water were found to be -156‰ (ranging from -133 to -192‰) for $\epsilon_{n-alkane/leaf-water}$ and +27.3‰ (ranging from +23.0 to +32.3‰) for $\epsilon_{sugar/leaf-water}$.
- (iii) Using measured leaf water isotope composition ($\delta^2H_{leaf-water}$ and $\delta^{18}O_{leaf-water}$) in a less (Eq. 6) and a more simplified rearranged Craig-Gordon model (Eq. 7), RH_{air} and RH_{leaf} can be derived, by using either T_{air} or T_{leaf} . Most accurately, the RH_{air} values via Eq. 7 can be reconstructed, with a calculated R^2 of 0.84 ($p < 0.001$) between measured and modeled RH_{air} and a RMSE of 6%. RH_{leaf} reconstructions seemed less robust.
- (iv) Reconstructed source water isotope composition (δ^2H_s , $\delta^{18}O_s$) are in range with the measured tank water ($\delta^2H_{tank-water}$, $\delta^{18}O_{tank-water}$). However, modeled δ^2H_s and $\delta^{18}O_s$ show a clear large range compared to $\delta^2H_{tank-water}$ and $\delta^{18}O_{tank-water}$. The uncertainties for source water determination are thus considerably higher compared to the relative humidity reconstructions. Still, the coupled δ^2H - $\delta^{18}O$ approach enables a back calculation of the plant source water. Uncertainties, with regard to relative humidity reconstructions via biomarker-based leaf water isotope composition, arose from leaf water reconstructions and model uncertainties, as shown in conclusions ii) and iii). Overall, the biomarker-based and measured RH_{air} correlation with a R^2 of 0.54 ($p < 0.001$) and a RMSE of 10% highlights the great potential of the coupled $\delta^2H_{n-alkane}$ - $\delta^{18}O_{sugar}$ paleohygrometer approach for reliable relative humidity reconstructions.

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Author contributions

J. Hepp and M. Zech wrote the paper; C. Mayr was responsible for the climate chamber experiment together with W. Stichler and provided the leaf samples and the data; M. Zech and R. Zech were responsible for compound-specific isotope analysis on the biomarkers; J. Hepp, M. Tuthorn and I. K. Schäfer did laboratory work and data evaluation of the biomarker compound-specific isotope analysis; B. Glaser, D. Juchelka, K. Rozanski and all co-authors contributed to the discussion and commented on the manuscript.

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Figure captions

Fig. 1: A: Plant water (leaf water, xylem water and soil water) isotope compositions (in green, orange and brown, respectively) and the isotope composition of the investigated leaf biomarkers (leaf wax n -alkanes n -C₂₉ and n -C₃₁ as open diamonds and triangles, respectively; hemicellulose-derived sugars: arabinose and xylose as open squares and circles, respectively) for the three plants *Eucalyptus globulus*, *Vicia faba* and *Brassica oleracea* grown in the climate chambers. B: Associated climate chamber conditions (leaf temperature and relative humidity in green and air temperature and relative humidity in red). Error bars represent analytical standard deviation of the respective measurements (see section 2.2 and Mayr, 2002).

Fig. 2: $\delta^2\text{H}$ - $\delta^{18}\text{O}$ diagram illustrating the isotope composition of the biomarkers, comprising $\delta^2\text{H}$ values of the leaf wax n -alkanes (C₂₉ for *Eucalyptus globulus* and *Brassica oleracea*; weighted mean of C₂₉ and C₃₁ for *Vicia faba*) and $\delta^{18}\text{O}$ values of the hemicellulose-derived sugars arabinose and xylose (black crosses) and the measured isotope compositions of leaf water (green squares), xylem water (orange squares), soil water (brown squares), atmospheric water vapor (red squares) and the tank water used for irrigation (blue triangle), which plot very close to the global meteoric water line.

Fig. 3: Scatterplots depicting the relationships between the compound-specific biomarker isotope composition and the respective leaf water values (A: $\delta^2\text{H}_{n\text{-alkane}}$ vs. $\delta^2\text{H}_{\text{leaf-water}}$; B: $\delta^{18}\text{O}_{\text{sugar}}$ vs. $\delta^{18}\text{O}_{\text{leaf-water}}$). *Brassica oleracea*, *Eucalyptus globulus* and *Vicia faba* samples are shown in purple, orange and black, respectively. Error bars of the δ values represent standard deviation of repeated measurements (see section 2.2 and Mayr, 2002).

Fig. 4: Boxplots comprising the plant-specific fractionation between the biomarkers and the leaf water (A: $\varepsilon_{n\text{-alkane/leaf-water}}$ according Eq. 8; B: $\varepsilon_{\text{sugar/leaf-water}}$ according to Eq. 9). *Brassica oleracea*, *Eucalyptus globulus* and *Vicia faba* samples are shown in purple, orange and black, respectively. Boxplots show median (thick black line), interquartile range (IQR) with upper (75%) and lower (25%) quartiles, lower and upper whiskers, which are restricted to $1.5 \cdot \text{IQR}$. Outside the $1.5 \cdot \text{IQR}$ space, the data points are marked with a dot. The notches extend to $\pm 1.58 \cdot \text{IQR} / \sqrt{n}$, by convention and give a 95% confidence interval for the difference of two medians (McGill et al., 1978).

Fig. 5: Scatterplots illustrating the correlation between leaf water-based and measured air/leaf relative humidity [modeled vs. measured RH_{air} (A) and RH_{leaf} (B)], modeled vs. measured leaf water deuterium-excess [T_{air} -based (B) and T_{leaf} -based (E) d_e vs. deuterium-excess_{leaf-water}] and modeled vs. measured LEL slopes [T_{air} -based (C) and T_{leaf} -based (F) vs. measured slopes]. In red, the results of the less simplified models are displayed (Eq. 2 for d_e , Eq. 6 for RH and Eq. 10 for S_{LEL}) and in black the results of the more simplified models are shown (Eq. 3 and d_e , Eq. 7 for RH and Eq. 11 for S_{LEL}). Black lines indicate the 1:1 relationship. R^2 and RMSE are calculated as described in section 2.4, while the RMSE values have the dimensions of the respective variables. Error bars for the measured RH values represent analytical standard deviations (see Mayr, 2002). For the uncertainties of the calculated and modeled variables see section 2.4.

Fig. 6: Boxplots showing the measured leaf water in comparison to the biomarker-based leaf water (according Eqs. 8 and 9), tank water, source water calculated with biomarker-based leaf water values and source water based on measured leaf water. Source water isotope compositions were calculated via the slopes of the LEL's (either with biomarker-based or measured leaf water values) and the GMWL.

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The numbers (1-4) mark the available scenarios for source water reconstruction (see section 2.4): 1 = S_{LEL} calculated according more simplified Eq. 11 with T_{air} , 2 = as 1 but with T_{leaf} , 3 = S_{LEL} calculated according less simplified Eq. 10 with T_{air} , 4 = as 3 but with T_{leaf} . Boxplots show median (thick black line), interquartile range (IQR) with upper (75%) and lower (25%) quartiles, lower and upper whiskers, which are restricted to $1.5 \cdot IQR$. Outside the $1.5 \cdot IQR$ space, the data points are marked with a dot. The notches are extend to $\pm 1.58 \cdot IQR / \sqrt{n}$, by convention and give a 95% confidence interval for the difference of two medians (McGill et al., 1978).

Fig. 7: Scatterplots depicting the relationship between biomarker-based (modeled) and measured air/leaf relative humidity [RH_{air} (A) and RH_{leaf} (B)]. Black lines indicate the 1:1 relationship. R^2 and RMSE was calculated as described in section 2.4, while the RMSE values have the dimensions of the respective variables. Error bars for the measured values represent analytical standard deviations (see Mayr, 2002). For uncertainty calculation of the modeled properties, see section 2.4. In addition, the leaf water-based air/leaf relative humidity results (from Fig. 5A and D) are shown in light colors for comparison.

Fig. S1: Boxplots comprising the plant-specific $\delta^2H_{n-alkane}$ (A) and $\delta^{18}O_{sugar}$ values (B). *Brassica oleracea*, *Eucalyptus globulus* and *Vicia faba* samples are shown in purple, orange and black, respectively. Boxplots show median (thick black line), interquartile range (IQR) with upper (75%) and lower (25%) quartiles, lower and upper whiskers, which are restricted to $1.5 \cdot IQR$. Outside the $1.5 \cdot IQR$ space, the data points are marked with a dot. The notches are extend to $\pm 1.58 \cdot IQR / \sqrt{n}$, by convention and give a 95% confidence interval for the difference of two medians (McGill et al., 1978).

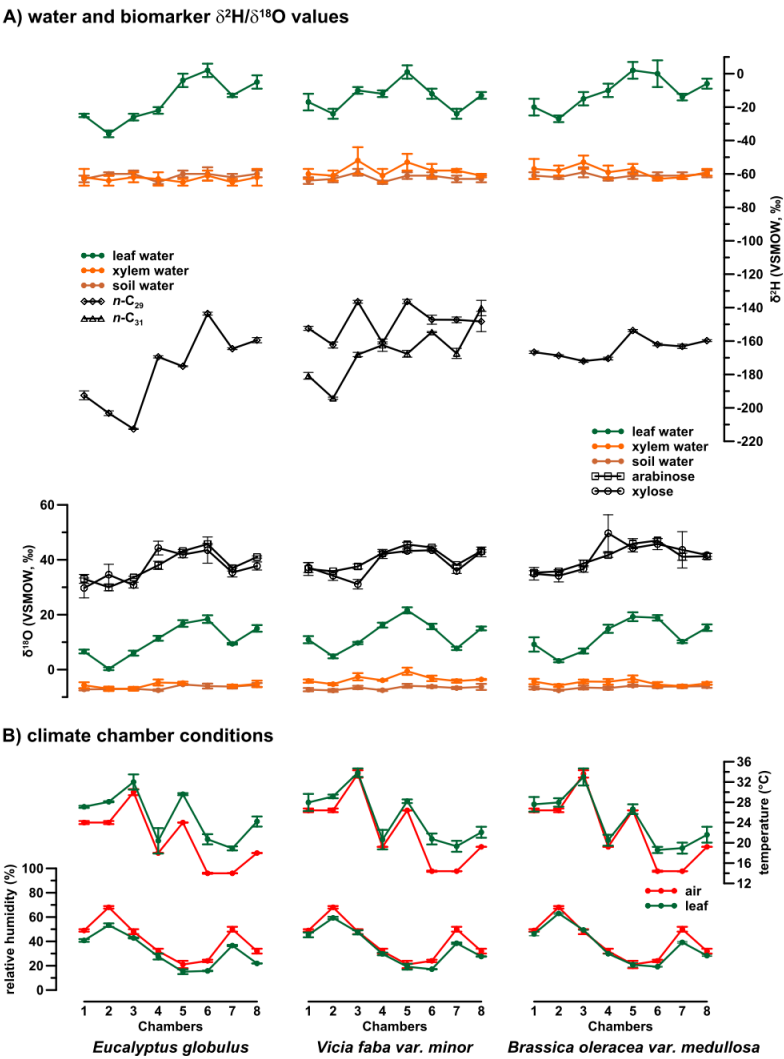
Fig. S2: Scatterplots of the fractionation between the biomarkers and leaf water vs. air temperature, air relative humidity (A and B: $\epsilon_{n-alkane/leaf-water}$ according Eq. 13; C and D $\epsilon_{sugar/leaf-water}$ according Eq. 14). *Brassica oleracea*, *Eucalyptus globulus* and *Vicia faba* samples are shown in purple, orange and black, respectively. Error bars for the measured values represent analytical standard deviations of repeated measurements (see section 2.2 and Mayr, 2002). For uncertainty calculation of the ϵ values, see section 2.4.

Fig. S3: Boxplots comprising measured and modeled RH (A) and deuterium-excess values (B). The numbers (1-2) mark the two available models for $RH_{leaf/air}$ and d_e reconstruction (see section 2.4): 1 = more simplified models (Eq. 3 for d_e and Eq. 7 for RH), 2 = less simplified models (Eq. 2 for d_e and Eq. 6 for RH). Boxplots show median (thick black line), interquartile range (IQR) with upper (75%) and lower (25%) quartiles, lower and upper whiskers, which are restricted to $1.5 \cdot IQR$. Outside the $1.5 \cdot IQR$ space, the data points are marked with a dot. The notches are extend to $\pm 1.58 \cdot IQR / \sqrt{n}$, by convention and give a 95% confidence interval for the difference of two medians (McGill et al., 1978).

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992 Fig. 1

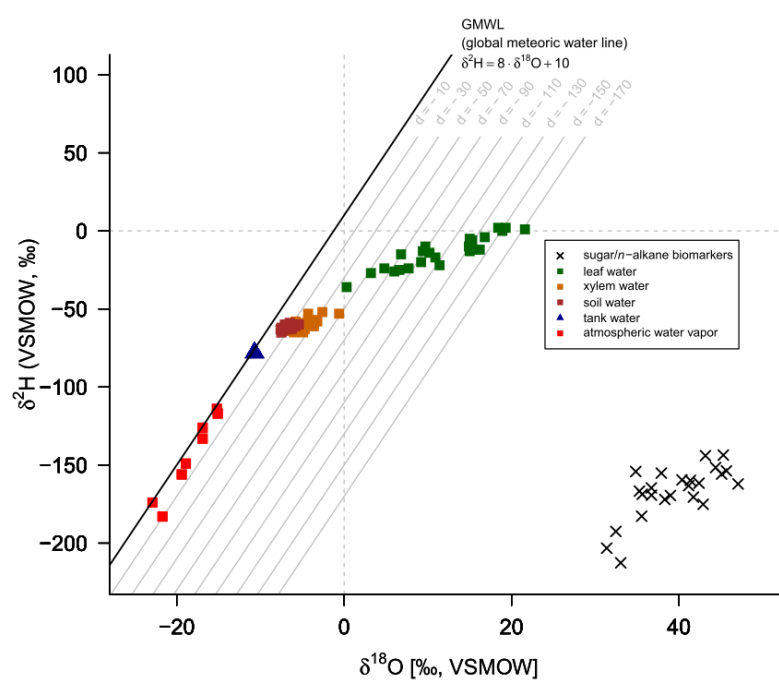


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994 Fig. 2

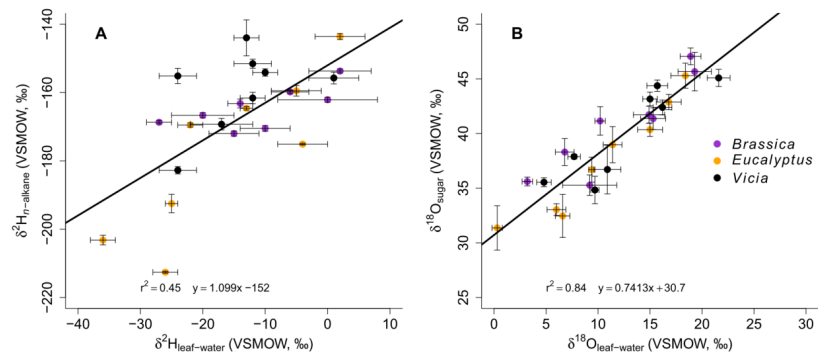


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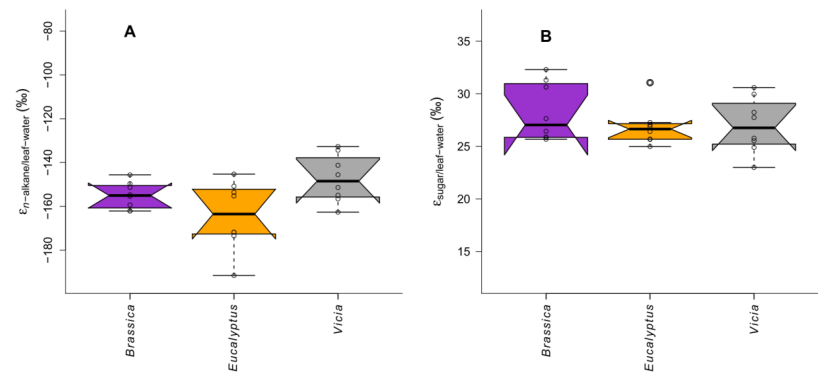
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997 Fig. 3



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999 Fig. 4



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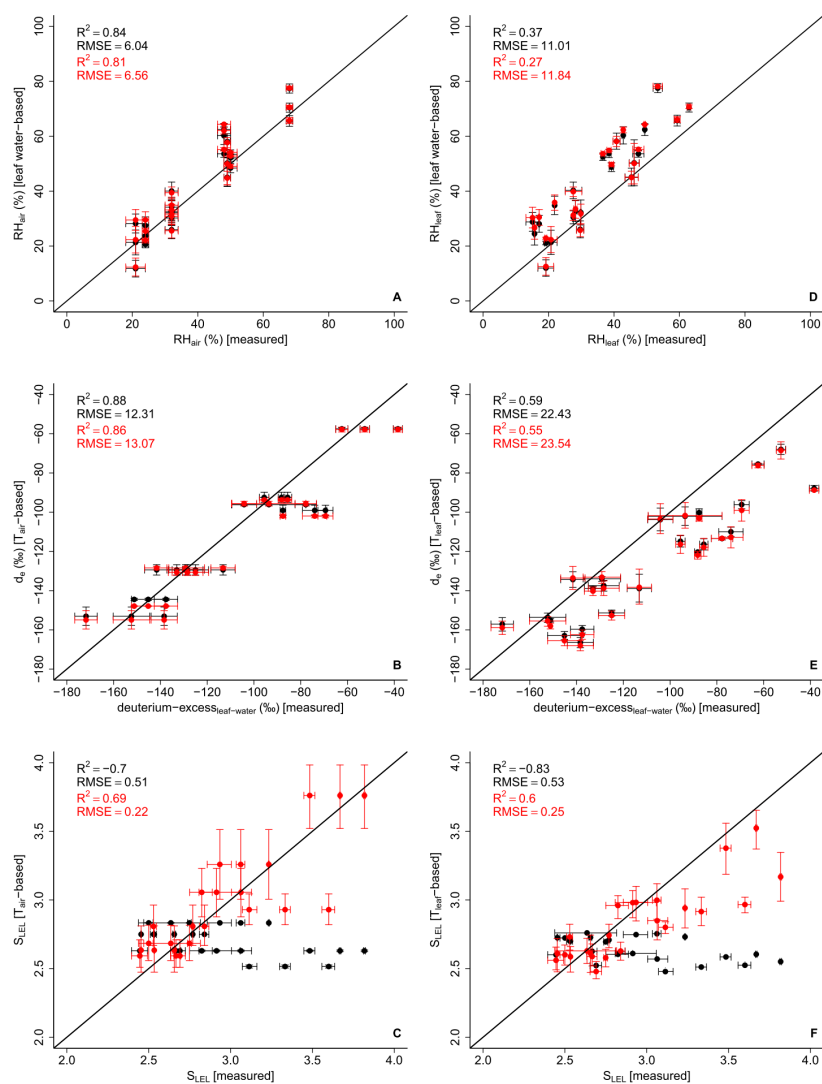
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1002 Fig. 5

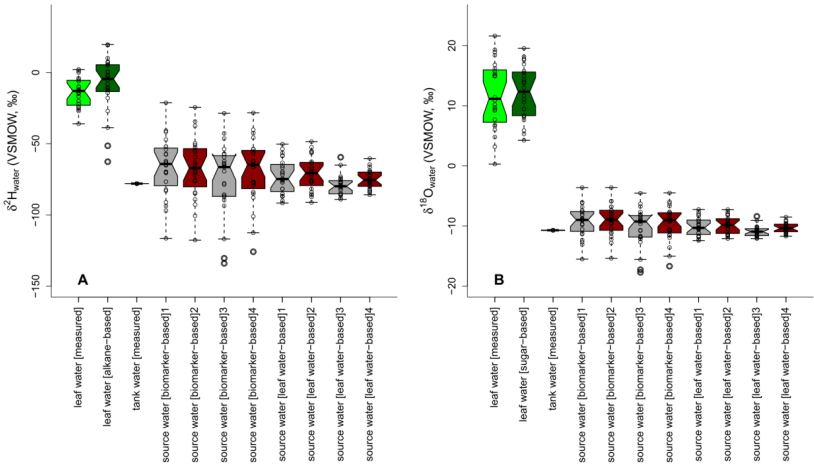


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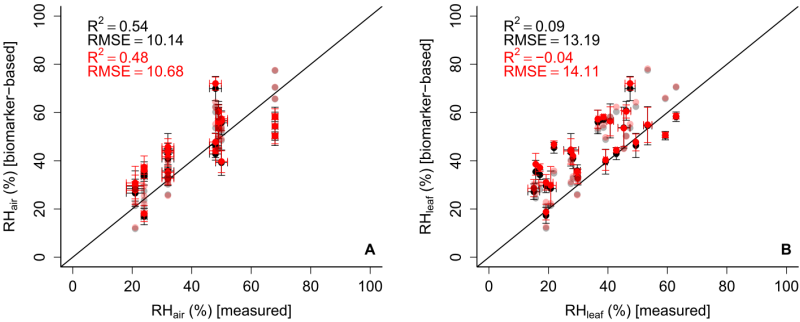
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1005 Fig. 6



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1007 Fig. 7

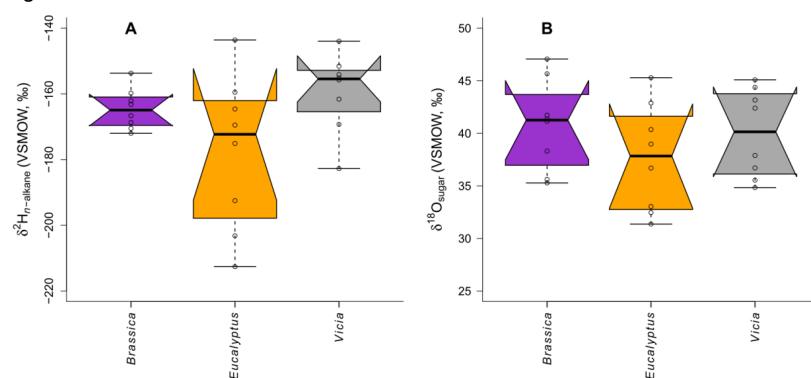


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1010 **Fig. S1**

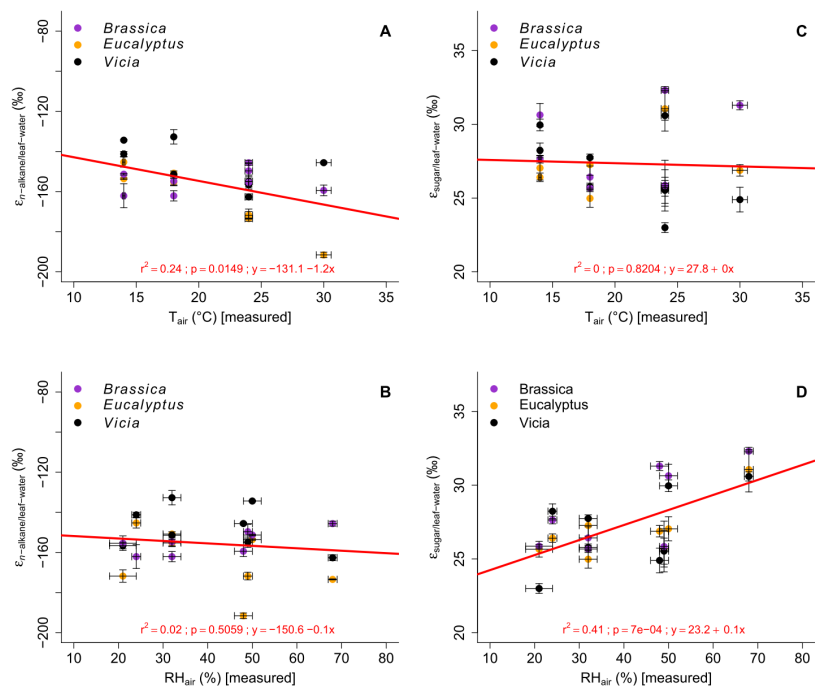


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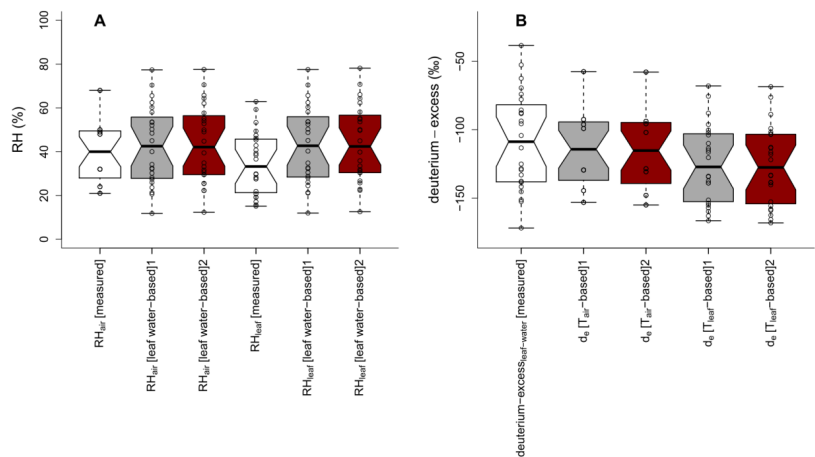
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1013 Fig. S2



1014 Fig. S3
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1 **Evaluation of bacterial glycerol dialkyl glycerol tetraether and ^2H -**
2 **^{18}O biomarker proxies along a Central European topsoil transect**

3 Johannes Hepp^{1,2,*}, Imke K. Schäfer³, Verena Lanny⁴, Jörg Franke³, Marcel
4 Bliedtner^{3,a}, Kazimierz Rozanski⁵, Bruno Glaser², Michael Zech^{2,6}, Timothy I.
5 Eglinton⁴, Roland Zech^{3,a}

6 ¹Chair of Geomorphology and BayCEER, University of Bayreuth, 95440 Bayreuth, Germany and

7 ²Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin-Luther-University
8 Halle-Wittenberg, 06120 Halle, Germany

9 ³Institute of Geography and Oeschger Centre for Climate Change Research, University of Bern, 3012
10 Bern, Switzerland

11 ⁴Department of Earth Science, ETH Zurich, 8092 Zurich, Switzerland

12 ⁵Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, 30-
13 059 Kraków, Poland

14 ⁶Institute of Geography, Faculty of Environmental Sciences, Technical University of Dresden, 01062
15 Dresden, Germany

16 ^anow at Institute of Geography, Chair of Physical Geography, Friedrich-Schiller University of Jena,
17 07743 Jena, Germany

18

19 *corresponding author (johannes-hepp@gmx.de)

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20 **Keywords**

21 Leaf wax *n*-alkanes, hemicellulose sugars, pH, temperature, CBT, MBT', precipitation
 22 $\delta^2\text{H}/\delta^{18}\text{O}$, relative humidity

23 **Abstract**

24 Molecular fossils, like bacterial branched glycerol dialkyl glycerol tetraethers (brGDGTs), and
 25 the stable isotopic composition of biomarkers, such as $\delta^2\text{H}$ of leaf wax-derived *n*-alkanes ($\delta^2\text{H}_n$ -
 26 alkane) or $\delta^{18}\text{O}$ of hemicellulose-derived sugars ($\delta^{18}\text{O}_{\text{sugar}}$) are increasingly used for the
 27 reconstruction of past climate and environmental conditions. Plant-derived $\delta^2\text{H}_{n\text{-alkane}}$ and
 28 $\delta^{18}\text{O}_{\text{sugar}}$ values record the isotopic composition of plant source water ($\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$),
 29 which usually reflects mean annual precipitation ($\delta^2\text{H}/\delta^{18}\text{O}_{\text{precipitation}}$), modulated by
 30 evapotranspirative leaf water enrichment and biosynthetic fractionation. Accuracy and
 31 precision of respective proxies should be ideally evaluated at a regional scale. For this study,
 32 we analysed topsoils below coniferous and deciduous forests, as well as grassland soils along a
 33 Central European transect in order to investigate the variability and robustness of various
 34 proxies, and to identify effects related to vegetation. Soil pH-values derived from brGDGTs
 35 correlate reasonably well with measured soil pH-values, but systematically overestimate them
 36 ($\Delta\text{pH} = 0.6 \pm 0.6$). The branched vs. isoprenoid tetraether index (BIT) can give some indication
 37 whether the pH reconstruction is reliable. Temperatures derived from brGDGTs overestimate
 38 mean annual air temperatures slightly ($\Delta T_{\text{MA}} = 0.5^\circ\text{C} \pm 2.4$). Apparent isotopic fractionation ($\epsilon_{n\text{-alkane/precipitation}}$
 39 and $\epsilon_{\text{sugar/precipitation}}$) is lower for grassland sites than for forest sites due to “signal
 40 damping”, i.e. grass biomarkers do not record the full evapotranspirative leaf water enrichment.
 41 Coupling $\delta^2\text{H}_{n\text{-alkane}}$ with $\delta^{18}\text{O}_{\text{sugar}}$ allows to reconstruct the stable isotopic composition of the
 42 source water more accurately than without the coupled approach ($\Delta\delta^2\text{H} = \sim 21\text{‰} \pm 22$ and
 43 $\Delta\delta^{18}\text{O} = \sim 2.9\text{‰} \pm 2.8$). Similarly, relative humidity during daytime and vegetation period
 44 (RH_{MDV}) can be reconstructed using the coupled isotope approach ($\Delta\text{RH}_{\text{MDV}} = \sim 17 \pm 12$).
 45 Especially for coniferous sites, reconstructed RH_{MDV} values as well as source water isotope
 46 composition underestimate the measured values. This can be likely explained by understory
 47 grass vegetation at the coniferous sites contributing significantly to the *n*-alkane pool but only
 48 marginally to the sugar pool in the topsoil. The large uncertainty likely reflect the fact that
 49 biosynthetic fractionation is not constant, as well as microclimate variability. Overall, GDGTs
 50 and the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach have great potential for more quantitative
 51 paleoclimate reconstructions.

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52 1 Introduction

53 Information about the variability and consequences of past climate changes is a prerequisite for
 54 precise predictions regarding the present climate change. Molecular fossils, so called
 55 biomarkers, climate proxies have great potential to enhance our understanding about variations
 56 of past climate and environmental changes. Lipid biomarkers in particular, are increasingly
 57 used for paleoclimate and environmental reconstructions (e.g. Brincat et al., 2000; Eglinton and
 58 Eglinton, 2008; Rach et al., 2014; Romero-Viana et al., 2012; Schreuder et al., 2016). However
 59 strengths and limitations of respective proxies need known (Dang et al., 2016). For this,
 60 calibrations using modern reference samples are essential.

61 Terrestrial branched glycerol dialkyl glycerol tetraethers (brGDGTs) that are synthesized in the
 62 cell membranes of anaerobe heterotrophic soil bacteria (Oppermann et al., 2010; Weijers et al.,
 63 2010) have great potential for the reconstruction of past environmental conditions (e.g. Coffinet
 64 et al., 2017; Schreuder et al., 2016; Zech et al., 2012), although some uncertainties exist.
 65 Calibration studies suggest that the relative abundance of the individual brGDGTs varies with
 66 mean annual air temperature (T_{MA}) and soil pH (Peterse et al., 2012; Weijers et al., 2007), at
 67 least across large, global climate gradients or along pronounced altitudinal gradients (Wang et
 68 al., 2017). However, in arid regions the production of brGDGT is limited, while isoprenoidal
 69 GDGTs (iGDGTs) produced by archaea provide the dominant part of the overall soil GDGT
 70 pool (Anderson et al., 2014; Dang et al., 2016; Dirghangi et al., 2013; Wang et al., 2013; Xie
 71 et al., 2012). The ratio of brGDGTs vs. isoprenoid GDGTs (BIT) can be used as indication
 72 whether a reconstruction of T_{MA} and pH will be reliable. Moreover, Mueller-Niggemann et al.
 73 (2016) revealed an influence of the vegetation cover on the brGDGT producing soil microbes.
 74 From field experiments, it is known, that vegetation type and mulching practice strongly effect
 75 soil temperature and moisture (Awe et al., 2015; Liu et al., 2014). Thus, multiple factors can be
 76 expected to influence soil microbial communities and GDGT production. So far, little is known
 77 about the variability of GDGT proxies on a regional scale, and a calibration study with small
 78 climate gradient but with different vegetation types might be useful.

79 Compound specific stable hydrogen isotopes of leaf wax biomarkers, such as long chain n -
 80 alkanes ($\delta^2H_{n-alkanes}$) record the isotopic signal of precipitation and therefore past climate and
 81 environmental conditions (Sachse et al., 2004, 2006). However, various influencing factors are
 82 known all along the way from the moisture source to leaf waxes (Pedentchouk and Zhou, 2018
 83 and Sachse et al., 2012 for review). One is the evapotranspiration of leaf water (Feakins and
 84 Sessions, 2010; Kahmen et al., 2013; Zech et al., 2015), which is strongly driven by relative air
 85 humidity (RH; e.g. Cernusak et al., 2016 for review). In addition, a strong precipitation signal
 86 is known to be incorporated into long chain leaf waxes (Hou et al., 2008; Rao et al., 2009;
 87 Sachse et al., 2004). In paleoclimate studies, it is often not feasible to disentangle between the
 88 evapotranspirative enrichment from the precipitation signal. Zech et al. (2013) proposed to
 89 couple $\delta^2H_{n-alkane}$ results with oxygen stable isotopes of hemicellulose-derived sugars ($\delta^{18}O_{sugar}$).
 90 Assuming constant biosynthetic fractionation factors (ϵ_{bio}) for the different compound classes
 91 (n -alkanes and hemicellulose sugars), the coupling enables the reconstruction of the isotopic
 92 composition of leaf water, RH and $\delta^2H/\delta^{18}O$ of plant source water ($\approx \delta^2H/\delta^{18}O$ of precipitation;
 93 Tuthorn et al., 2015). So far, a detailed evaluation of this approach on the European scale, as
 94 well as concerning possible effects related to vegetation changes is missing.

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We analysed topsoil samples under coniferous, deciduous and grassland vegetation along a Central European transect in order to estimate the variability of the biomarker proxies. More specifically, we aim to test whether:

(i) the vegetation type has an influence on the brGDGT proxies, the $\delta^2\text{H}_{n\text{-alkane}}$ and the $\delta^{18}\text{O}_{\text{sugar}}$ stable isotopic composition, as well as on reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ and RH.

(ii) the published brGDGT proxies used for reconstructing mean annual temperature and soil pH are sensitive enough to reflect the medium changes in temperature and soil pH along our transect.

(iii) the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ approach faithfully reflects $\delta^2\text{H}/\delta^{18}\text{O}$ of precipitation and RH along the transect.

105

2 Material and methods

2.1 Geographical setting and sampling

In November 2012, we collected topsoil samples (0-5 cm depth) at 16 locations along a transect from Southern Germany to Southern Sweden (Fig. 1A) and distinguished between sites with coniferous forest (con, $n = 9$), deciduous forest (dec, $n = 14$) and grassland (grass, $n = 6$) vegetation cover (for more details see Schäfer et al. (2016) and Tab. S1).

112

2.2 Database of instrumental climate variables and isotope composition of precipitation

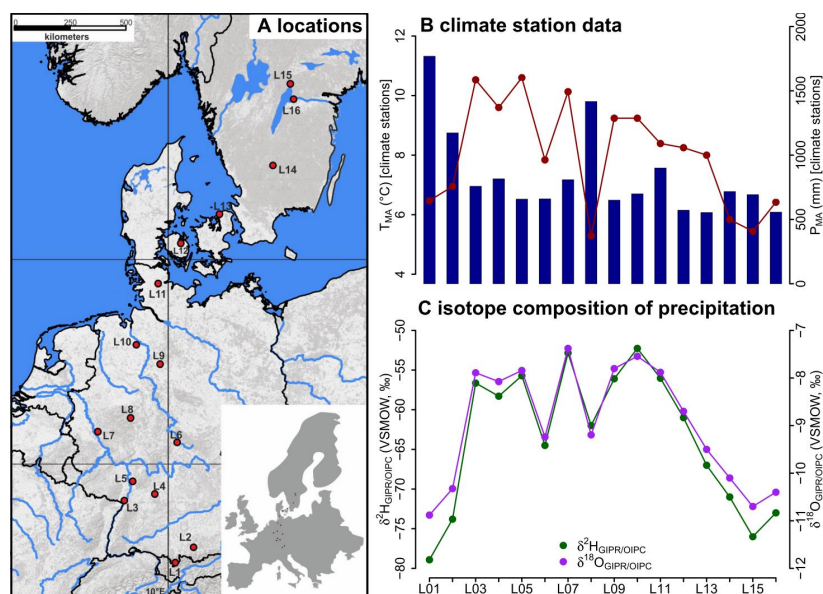
Climate data was derived from close-by weather observation stations operating by the regional institutions (Deutscher Wetterdienst (DWD) for Germany, Danmarks Meteorologiske Institut (DMI) for Denmark and the Sveriges Meteorologiska och Hydrologiska Institute (SMHI) for Sweden). The DWD provides hourly data for each station (DWD Climate Data Center, 2018b), enabling not only the calculation of T_{MA} , but also of the mean annual relative air humidity (RH_{MA}), mean temperature and relative air humidity during the vegetation period (T/RH_{MV}), and of daytime temperature and relative humidity averages over the vegetation period (T/RH_{MDV}). In addition, annual precipitation observations were used to derive the mean annual precipitation amount (P_{MA} ; DWD Climate Data Center, 2018b). From the DMI, the respective climate variables were derived from published technical reports (Cappelen, 2002; Frich et al., 1997; Laursen et al., 1999). The SMHI provides open data from which we derived the climate variables for the Swedish sites (Swedish Meteorological and Hydrological Institute, 2018). For more details about the climate database used for calculations and comparisons, the reader is referred to Tab. S2.

For comprising German precipitation $\delta^2\text{H}/\delta^{18}\text{O}$ along the transect, we realized a regionalisation (called $\delta^2\text{H}/\delta^{18}\text{O}_{\text{GIPR}}$) using online available data from 34 German GNIP stations, 4 Austrian ANIP stations and the Groningen GNIP station (van Geldern et al., 2014; IAEA/WMO, 2018; Stumpp et al., 2014; Umweltbundesamt GmbH, 2018), following the approach of Schlotter (2007). However, instead of the multivariate regression procedure applied by Schlotter (2007), we used a random forest approach (Hothorn et al., 2006; Strobl et al., 2007, 2008) to describe the relationship of squared latitude, longitude and altitude vs. long term weighted means of precipitation $\delta^2\text{H}/\delta^{18}\text{O}$, and realized the prediction for the study sites. For the Danish

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136 and Swedish sites, such a procedure was not possible. Hence, the annual precipitation $\delta^2\text{H}/\delta^{18}\text{O}$
 137 values were derived from the Online Isotopes in Precipitation Calculator (OIPC, version 3.1),
 138 therefore called $\delta^2\text{H}/\delta^{18}\text{O}_{\text{OIPC}}$ (Bowen, 2018; Bowen and Revenaugh, 2003; IAEA/WMO,
 139 2015). The finally used $\delta^2\text{H}/\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ data are given in Tab. S1.
 140 The T_{MA} along the transect ranges from 5.3 to 10.6°C, and P_{MA} ranges from 554 to 1769 mm
 141 (Fig. 1B). Precipitation $\delta^2\text{H}/\delta^{18}\text{O}$ shows moderate changes along the transect, $\delta^2\text{H}_{\text{GIPR/OIPC}}$
 142 varies between -52 and -79‰, and $\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ ranges from -7.4 to -10.9‰ (Fig. 1C).
 143 Correlations between $\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ and P_{MA} , altitude of the locations, T_{MA} are given in the
 144 supplementary material (Fig. S1 to S3), along with a $\delta^2\text{H}_{\text{GIPR/OIPC}}$ vs. $\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ scatter plot
 145 (Fig. S4).



146 **Fig. 1.** (A) Sample locations (red dots, map source: US National Park Service), (B) variations
 147 of mean annual air temperature (T_{MA}) and mean annual precipitation (P_{MA}) derived from close-
 148 by climate station data, and (C) hydrogen and oxygen stable isotope composition of
 149 precipitation ($\delta^2\text{H}_{\text{GIPR/OIPC}}$ and $\delta^{18}\text{O}_{\text{GIPR/OIPC}}$, respectively) as derived for the sampled transect
 150 locations (see section 2.2 GIPR $\delta^2\text{H}/\delta^{18}\text{O}$ generation procedure). The reader is referred to
 151 section 2.2 (and Tab. S1 and S2) for database and reference information of data plotted in (B)
 152 and (C).
 153

154

155 2.3 Soil extractions and analysis

156 2.3.1 GDGTs and pH

157 A detailed description of sample preparation for lipid analysis can be found in Schäfer et al.
 158 (2016). Briefly, 1–6 g freeze-dried and grounded soil sample was microwave extracted with 15

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ml dichloromethane (DCM)/methanol (MeOH) 9:1 (v:v) at 100°C for 1 h. Extracts were separated over aminopropyl silica gel (Supelco, 45 µm) pipette columns. The nonpolar fraction (including *n*-alkanes) was eluted with hexane and further purified over AgNO₃ coated silica pipette columns (Supelco, 60-200 mesh) and zeolite (Geokleen Ltd.). The GDGT-containing fraction was eluted with DCM:MeOH 1:1 (v:v), re-dissolved in hexane/isopropanol (IPA) 99:1 (v:v) and transferred over 0.45 µm PTFE filters into 300 µl inserts. For quantification, a known amount of a C₄₆ diol standard was added after transfer. The samples were analysed at ETH Zurich using an Agilent 1260 Infinity series HPLC–atmospheric chemical pressure ionization mass spectrometer (HPLC–APCI-MS) equipped with a Grace Prevail Cyano column (150 mm × 2.1 mm; 3 µm). The GDGTs were eluted isocratically with 90% A and 10% B for 5 min and then with a linear gradient to 18% B for 34 min at 0.2 ml min⁻¹, where A=hexane and B=hexane/isopropanol (9:1, v:v). Injection volume was 10 µl and single ion monitoring of [M+H]⁺ was used to detect GDGTs.

The pH of the samples was measured in the laboratory of the Soil Biogeochemistry group, Institute of Agronomy and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, in a 1:3 soil:water (w/v) mixture.

175

2.3.2 δ²H_{*n*-alkane}

The hydrogen isotopic composition of the highest concentrated *n*-alkanes (*n*-C₂₅, *n*-C₂₇, *n*-C₂₉, *n*-C₃₁, and *n*-C₃₃) was determined using a TRACE GC Ultra Gas Chromatography connected to a Delta V Plus Isotope Ratio Mass Spectrometer via a ²H pyrolysis reactor (GC-²H-Py-IRMS; Thermo Scientific, Bremen, Germany) at the ETH Zurich. The compound-specific ²H/¹H ratios were calibrated against an external standard with C₁₅ – C₃₅ homologues. External standard mixtures (A4 mix from A. Schimmelmann, University of Indiana) were run between the samples for multipoint linear normalization. The H₃ factor was determined on each measurement day and was constant throughout the periods of the sample batches. Samples were analysed in duplicates, and results typically agreed within 4% (average difference = 1.4%). All δ²H values are expressed relative to the Vienna Standard Mean Ocean Water (V-SMOW).

187

2.3.3 δ¹⁸O_{sugar}

Hemicellulose sugars were extracted and purified using a slightly modified standard procedure (Amelung et al., 1996; Guggenberger et al., 1994; Zech and Glaser, 2009). Briefly, myoinositol was added to the samples prior to extraction as first internal standard. The sugars were released hydrolytically using 4M trifluoroacetic acid for 4 h at 105°C, cleaned over glass fiber filters and further purified using XAD and Dowex columns. Before derivatization with methylboronic acid (Knapp, 1979), the samples were frozen, freeze-dried, and 3-O-methylglucose in dry pyridine was added as second internal standard. Compound-specific hemicellulose sugar ¹⁸O measurements were performed in the laboratory of the Soil Biogeochemistry group, Institute of Agronomy and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, using GC-¹⁸O-Py-IRMS (all devices from Thermo Fisher Scientific, Bremen, Germany). Standard deviations of the triplicate measurements were 1.4‰ (over 29 investigated samples) for arabinose and xylose, respectively. We focus on these two hemicellulose-derived neutral sugars

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arabinose and xylose as they strongly predominate over fucose in terrestrial plants, soils and sediments (Hepp et al., 2016 and references therein). Rhamnose concentrations were too low to obtain reliable $\delta^{18}\text{O}$ results. All $\delta^{18}\text{O}$ values are expressed relative to the Vienna Standard Mean Ocean Water (V-SMOW).

205

2.4 Theory and Calculations

2.4.1 Calculations used for the GDGT-based reconstructions

The branched and isoprenoid tetraether (BIT) index is calculated according to Hopmans et al. (2004), for structures see Fig. S5:

$$\text{BIT} = \frac{\text{Ia} + \text{IIa} + \text{IIIa}}{\text{Ia} + \text{IIa} + \text{IIIa} + \text{crenarchaeol}} \quad (1)$$

The cyclopentane moiety number of brGDGTs correlates negatively with soil pH (Weijers et al., 2007), which led to the development of the cyclization of branched tetraethers (CBT) ratio. CBT and the CBT based pH (pH_{CBT}) were calculated according to Peterse et al. (2012):

$$\text{CBT} = -\log \frac{\text{Ib} + \text{IIb}}{\text{Ia} + \text{IIa}}, \quad (2)$$

$$\text{pH}_{\text{CBT}} = 7.9 - 1.97 \times \text{CBT}. \quad (3)$$

The number of methyl groups in brGDGTs correlates negatively with T_{MA} and soil pH (Peterse et al., 2012; Weijers et al., 2007). Thus, the ratio of the methylation of branched tetraethers (MBT) ratio and the CBT ratio can be used to reconstruct T_{MA} . We use the equation given by Peterse et al. (2012):

$$\text{MBT}' = \frac{\text{Ia} + \text{Ib} + \text{Ic}}{\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa} + \text{IIb} + \text{IIc} + \text{IIIa}}, \quad (4)$$

$$T_{\text{MA}} = 0.81 - 5.67 \times \text{CBT} + 31.0 \times \text{MBT}'. \quad (5)$$

222

2.4.2 Calculations and concepts used for the coupled $\delta^2\text{H}$ - $\delta^{18}\text{O}$ approach

The apparent fractionation is calculated according to Cernusak et al. (2016):

$$\varepsilon_{\text{n-alkane/precipitation}} = \left(\frac{\delta^2\text{H}_{\text{n-alkane}} - \delta^2\text{H}_{\text{GIPR/OIPC}}}{1 + \delta^2\text{H}_{\text{GIPR/OIPC}}/1000} \right), \quad (6)$$

$$\varepsilon_{\text{sugar/precipitation}} = \left(\frac{\delta^{18}\text{O}_{\text{sugar}} - \delta^{18}\text{O}_{\text{GIPR/OIPC}}}{1 + \delta^{18}\text{O}_{\text{GIPR/OIPC}}/1000} \right). \quad (7)$$

The isotopic composition of leaf water ($\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf water}}$) can be calculated using ε_{bio} for $\delta^2\text{H}_{\text{n-alkane}}$ (-160‰, Sachse et al., 2012; Sessions et al., 1999) and $\delta^{18}\text{O}_{\text{sugar}}$ (+27‰, Cernusak et al., 2003; Schmidt et al., 2001):

$$\delta^2\text{H}_{\text{leaf water}} = \left(\frac{1000 + \delta^2\text{H}_{\text{n-alkane}}}{1000 + \varepsilon_{\text{bio}} (\text{n-alkane})} \right) \times 10^3 - 1000, \quad (8)$$

$$\delta^{18}\text{O}_{\text{leaf water}} = \left(\frac{1000 + \delta^{18}\text{O}_{\text{sugar}}}{1000 + \varepsilon_{\text{bio}} (\text{sugar})} \right) \times 10^3 - 1000. \quad (9)$$

Zech et al. (2013) introduced the conceptual model for the coupled $\delta^2\text{H}_{\text{n-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ approach in detail. Briefly, the coupled approach is based on the following assumptions (illustrated in Fig. 8): (i) The isotopic composition of precipitation, which is set to be equal to the plant source water, typically plots along the global meteoric water line (GMWL; $\delta^2\text{H} = 8 \times \delta^{18}\text{O} + 10$) in a

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$\delta^{18}\text{O}$ vs. $\delta^2\text{H}$ space (Craig, 1961); (ii) Source water uptake by plants does not lead to any fractionation (e.g. Dawson et al., 2002), and significant evaporation of soil water can be excluded; (iii) Evapotranspiration leads to enrichment of the remaining leaf water along the local evaporation line (LEL; Allison et al., 1985; Bariac et al., 1994; Walker and Brunel, 1990), compared to the source water taken up by the plant; (iv) The biosynthetic fractionation is assumed to be constant. In addition, isotopic equilibrium between plant source water (~ weighted mean annual precipitation) and the local atmospheric water vapour is assumed. Further assumption concerns the isotope steady-state in the evaporating leaf water reservoir. The coupled approach allows for reconstructing the isotopic composition of plant source water ($\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$) from the reconstructed leaf water, by calculating the intercepts of the LELs with the GMWL (Zech et al., 2013). The slope of the LEL (S_{LEL}) can be assessed by the following equation (Gat, 1971):

$$S_{\text{LEL}} = \frac{\varepsilon_2^* + C_k^2}{\varepsilon_{18}^* + C_k^{18}}, \quad (10)$$

where ε^* are equilibrium isotope fractionation factors and C_k are kinetic fractionation factors. The latter equals to 25.1‰ and 28.5‰, for C_k^2 and C_k^{18} , respectively (Merlivat, 1978). The equilibrium fractionation factors can be derived from empirical equations (Horita and Wesolowski, 1994) by using T_{MDV} values. For two Danish sites T_{MDV} are not available, instead T_{MV} is used here (section 2.2 and Tab. S2).

In a $\delta^{18}\text{O}$ - $\delta^2\text{H}$ diagram, the distance of the leaf water from the GMWL define the deuterium-excess of leaf water ($d_{\text{leaf-water}} = \delta^2\text{H}_{\text{leaf-water}} - 8 \times \delta^{18}\text{O}_{\text{leaf-water}}$, according Dansgaard, (1964); Fig. 8). To convert $d_{\text{leaf-water}}$ into mean RH during daytime and vegetation period (RH_{MDV}), a simplified Craig-Gordon model can be applied (Zech et al., 2013):

$$\text{RH} = 1 - \frac{\Delta d}{\varepsilon_2^* - 8 \times \varepsilon_{18}^* + C_k^2 - 8 \times C_k^{18}}, \quad (11)$$

where Δd is the difference in $d_{\text{leaf-water}}$ and the deuterium-excess of source water ($d_{\text{source-water}}$).

2.5 Statistics

In the statistical analysis we checked sample distributions for normality (Shapiro and Wilk, 1965) and for equal variance (Levene, 1960). If normality and equal variances are given, we perform an Analysis of Variance (ANOVA). If that is not the case, we conduct the non-parametric Kruskal-Wallis Test. ANOVA or Kruskal-Wallis are used to find significant differences ($\alpha=0.05$) between the vegetation types (deciduous, conifer and grass).

In order to describe the relation along a 1:1 line, the coefficient of correlation (R^2) was calculated as $R^2 = 1 - \frac{\sum(\text{modeled} - \text{measured})^2}{\sum(\text{measured} - \text{measured mean})^2}$. The small r^2 is taken as coefficient of correlation of a linear regression between a dependent (y) and explanatory variable(s). The root mean square error (RMSE) of the relationships was calculated

as $\text{RMSE} = \sqrt{\left(\frac{1}{n} \cdot \sum(\text{modeled} - \text{measured})^2\right)}$. All data plotting and statistical analysis was realized in R (version 3.2.2; R Core Team, 2015).

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3 Results and Discussion

3.1 GDGT concentrations

GDGT Ia has the highest concentration under all vegetation types, followed by GDGT IIa and GDGT IIIa (Fig. 2). GDGT Ib, IIb and Ic occur in minor, GDGT IIc and IIIb only in trace amounts. GDGT IIIc was below the detection limit in most of the samples (Tab. S3). Although other studies document an influence of the vegetation cover on soil temperature and soil water content, which control the microbial community composition in soils (Awe et al., 2015; Liu et al., 2014; Mueller-Niggemann et al., 2016), we find no statistically different pattern of the individual brGDGTs.

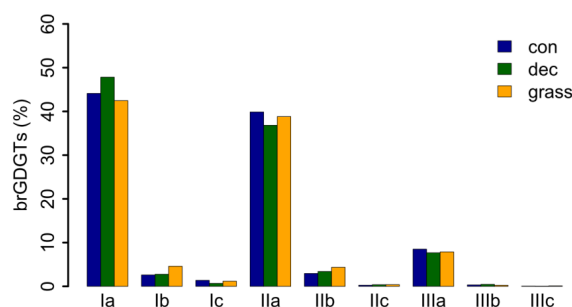


Fig. 2. Mean concentrations of individual brGDGTs as percentage of all brGDGTs for the three investigated types. Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest sites (n=14); grass = grassland sites (n=6).

Total concentrations of brGDGTs range from 0.32 to 9.17 $\mu\text{g/g}$ dry weight and tend to be highest for the coniferous samples and lowest for the grasses (Fig. 3A, Tab. S3). Bulk brGDGT concentrations lie within ranges of other studies examining soils of mid latitude regions (Huguet et al., 2010b, 2010a; Weijers et al., 2011). Similar concentrations in coniferous and deciduous samples imply that brGDGT production does not strongly vary in soils below different forest types. The grass samples show lower brGDGT concentrations compared to the forest samples, but this is probably mainly due to ploughing of the grass sites and hence admixing of mineral subsoil material. Anyhow, the differences in brGDGT concentrations are not significant (p-value = 0.06).

3.2 BIT index

Most of the samples have a BIT index higher than 0.9 (Fig 3B and Tab. S3). The BIT-values are typical for soils in humid and temperate climate regions (Weijers et al., 2006). However, outliers exist. The most likely source of iGDGTs in soils are Thaumarchaeota, i.e. aerobic ammonia oxidizing archaea producing Crenarchaeol and its regioisomer (Schouten et al., 2013 and references therein), precipitation amounts drop below 700-800 mm (Dang et al., 2016; Dirghangi et al., 2013). The P_{MA} data of our sampling sites mostly show precipitation > 550 mm (Fig. 1B), but one has to be aware that this data is based on the climate station nearest to the respective sampling locations and microclimate effects, such as sunlight exposure, canopy

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cover or exposition might have a pronounced influence on the brGDGT vs. iGDGT distribution. Mueller-Niggemann et al. (2016) found higher BIT indices in upland soils compared to paddy soils and stated that the management type also influences BIT values in soils. Along our transect, grass sites tend to have slightly lower BIT-values than forest sites, probably due to the absence of a litter layer and hence, no isolation mechanism preventing evaporation of soil water. Anyhow, differences between vegetation types are not significant (p -value = 0.32).

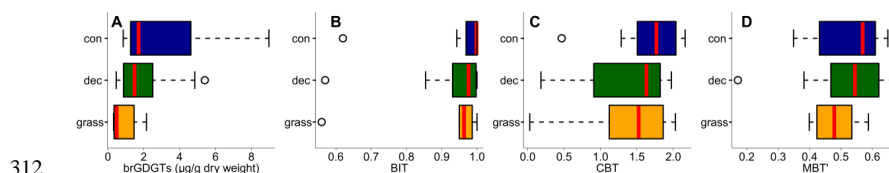


Fig. 3. (A) Total concentrations of brGDGTs in $\mu\text{g g}^{-1}$ dry weight, as well as (B) BIT, (C) CBT and (D) MBT. Abbreviations: con = coniferous forest sites ($n=9$); dec = deciduous forest sites ($n=14$); grass = grassland sites ($n=6$). Box plots show median (red line), interquartile range (IQR) with upper (75%) and lower (25%) quartiles, lowest whisker still within 1.5IQR of lower quartile, and highest whisker still within 1.5IQR of upper quartile, dots mark outliers.

3.3 CBT-derived pH

The CBT ratio shows a pronounced variation independent of vegetation type with values between 0.03 and 2.16 (Fig 3C). The coniferous samples tend to be highest, but the differences between vegetation types are not significant (p -value = 0.48). The CBT index can be related to pH in acidic and/or humid soils (e.g. Dirghangi et al., 2013; Mueller-Niggemann et al., 2016; Peterse et al., 2012; Weijers et al., 2007) but might be an indicator of soil water content and hence, precipitation in more arid and alkaline soils (e.g. Dang et al., 2016). There is a pronounced correlation between CBT and soil pH (Fig. 4), which is in good agreement with other studies from mid latitude regions where precipitation is relatively high (Anderson et al., 2014 and references therein). Moreover, the CBT to pH relationship in terms of slope and intersect in our dataset ($\text{CBT} = -0.47 \times \text{pH} + 3.5$, $r^2 = 0.7$, p -value < 0.0001, $n = 29$) is well comparable to the correlation described for the global calibration dataset of Peterse et al. (2012) ($\text{CBT} = -0.36 \times \text{pH} + 3.1$, $r^2 = 0.7$, p -value < 0.0001, $n = 176$).

However, there are some outliers in the CBT-pH correlation, which need a further examination (see locations grass L04, dec L10 and dec L12 as marked in Figs. 4 and 5). The outliers show lower BIT indices (< 0.85, Tab. S3). Even though the data from the nearest climate station suggest no abnormal P_{MA} . Local effects such as differences in the amount of sunlight exposure, nutrient availability for brGDGT producing organisms or, most likely soil water content might influence the brGDGT production at these locations (Anderson et al., 2014; Dang et al., 2016). A lower BIT index as well as a lower CBT occur when soil water content decreases (Dang et al., 2016; Sun et al., 2016) or when aeration is high and less anoxic microhabitats for GDGT producing microbes exist (e.g. Dirghangi et al., 2013).

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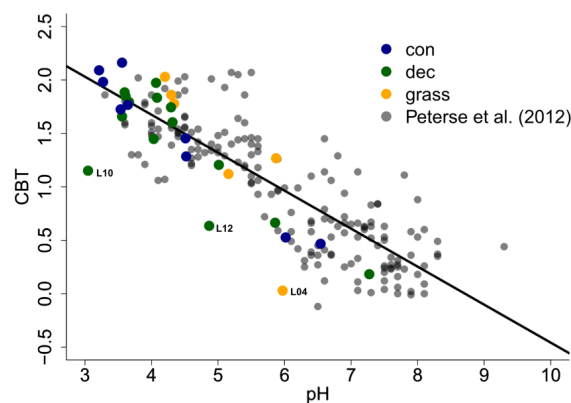


Fig. 4. CBT to pH relationship in our dataset in comparison to the global calibration dataset from Peterse et al. (2012) ($CBT = -0.36 \times pH + 3.1$, $r^2 = 0.7$, p -value < 0.0001 , $n = 176$, black line). Abbreviations: con = coniferous forest sites ($n=9$); dec = deciduous forest sites ($n=14$); grass = grassland sites ($n=6$).

As the CBT and pH are similarly correlated in our dataset and the global dataset of Peterse et al. (2012), the CBT-derived pH correlated well with the actual pH (Fig. 5A; $R^2 = 0.3$). Expressed as ΔpH (CBT-derived pH - measured pH), there is a tendency that the GDGTs result in an overestimation of the real pH for the forest sites (Fig. B). Yet a Kruskal-Wallis test shows no statistically significant difference between the vegetation types, with a p -value of 0.13. The overall ΔpH of 0.6 ± 0.6 shows that the reconstruction of soil pH using brGDGTs works well along this transect.

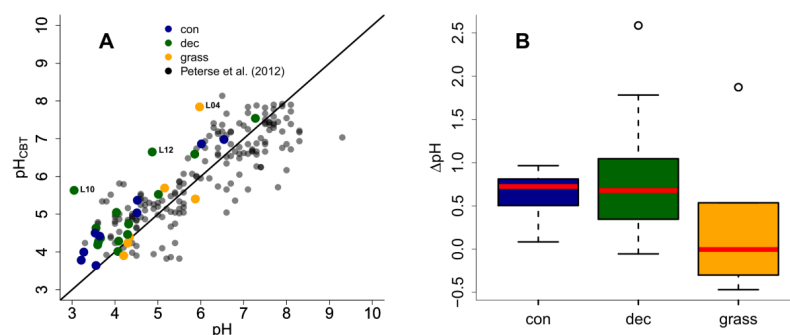


Fig. 5. (A) Correlation between measured pH and reconstructed soil pH (pH_{CBT}) from our transect data in comparison to the global calibration dataset from Peterse et al. (2012) ($R^2 = 0.7$, $RMSE = 0.75$, $n = 176$). Black line indicates the 1:1 relationship. (B) Boxplots of ΔpH (refers to $pH_{CBT} - pH$). Box plots show median (red line), interquartile range (IQR) with upper (75%)

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and lower (25%) quartiles, lowest whisker still within 1.5IQR of lower quartile, and highest whisker still within 1.5IQR of upper quartile, dots mark outliers. Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest sites (n=14); grass = grassland sites (n=6).

3.4 MBT'-CBT-derived T_{MA} reconstructions

The MBT' shows high variability with values ranging from 0.17 to 0.67 no statistical differences between vegetation types (p-value = 0.54; Fig. 3D, Tab. S3). When comparing reconstructed (MBT'-CBT-derived) T_{MA} with climate station T_{MA} , the data plot close to the 1:1 line, and fit well into the global dataset of Peterse et al. (2012) (Fig. 7A). The ΔT_{MA} reveal an overall offset of $0.5^{\circ}\text{C} \pm 2.4$ and there is no statistically difference between vegetation types (Fig. 7B). The standard deviation in ΔT_{MA} of ± 2.4 is well in line with the RMSE of 5.0 for the global calibration dataset (Peterse et al., 2012).

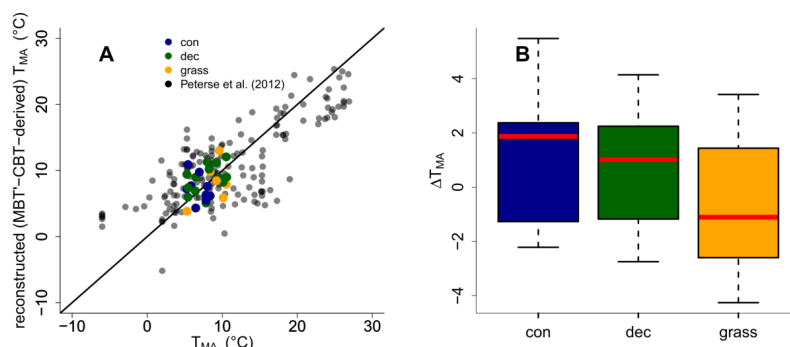


Fig. 6. (A) Correlation between climate station T_{MA} and reconstructed (MBT'-CBT-derived) T_{MA} . For comparison, the global calibration dataset from Peterse et al. (2012) is shown. The black line indicates the 1:1 relationship. (B) Boxplots of ΔT_{MA} (refers to reconstructed T_{MA} - T_{MA} from climate stations) in the different vegetation types from our transect study. Box plots show median (red line), interquartile range (IQR) with upper (75%) and lower (25%) quartiles, lowest whisker still within 1.5IQR of lower quartile, and highest whisker still within 1.5IQR of upper quartile, dots mark outliers. Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest sites (n=14); grass = grassland sites (n=6).

3.5 Apparent fractionation of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in the different vegetation types

The $\delta^2\text{H}$ values could be obtained for n -alkanes C_{27} , C_{29} and C_{31} in all samples and additionally at two locations for n - C_{25} and n - C_{33} at six other locations. The $\delta^2\text{H}_{n\text{-alkane}}$ values, calculated as mean of n - C_{25} to n - C_{31} $\delta^2\text{H}$, ranges from -156 to -216‰. Pooled standard deviations show an overall average of 3.6‰. The $\delta^{18}\text{O}_{\text{sugar}}$ values, calculated as the area weighted means for arabinose and xylose, ranges from 27.7 to 39.4‰. The average weighted mean standard deviation is 1.4‰. The compound-specific isotope data is summarized along with the calculations in Tab. S4.

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Apparent fractionation ($\epsilon_{n\text{-alkane/precipitation}}$) is on the order of -120 to -150‰, i.e. a bit less than the biosynthetic fraction of -160‰. This implies that evapotranspirative enrichment is ~ 10 to 40‰ (Fig. 7A). $\epsilon_{n\text{-alkane/precipitation}}$ is lower for grass sites compared to the forest sites. Differences are significant between deciduous and grass sites (p-value = 0.005). This finding supports the results of other studies (Kahmen et al., 2013; Liu and Yang, 2008; McInerney et al., 2011), and can be named “signal damping”. Grasses do not only incorporate the evaporatively-enriched leaf water only but also unenriched leaf water in the growth and differentiation zone of grasses (Gamarra et al., 2016; Liu et al., 2017).

The grass-derived hemicellulose sugar biomarkers do not fully record the evapotranspirative enrichment of the leaf water, either, as indicated by lower apparent fractionation ($\epsilon_{\text{sugar/precipitation}}$) in Fig. 7B. The differences are significant between forest and grass sites (p-value < 0.005). This is in agreement with a study on cellulose extracted from grass blades (Helliker and Ehleringer, 2002), and again, the “signal damping” can be explained with incorporation of enriched leaf water and non-enriched stem water.

Based on the comparison of evapotranspirative enrichment between forest and grass sites, the “signal damping” can be quantified to be ~ 31% for the hemicellulose sugars, and ~ 49% for the *n*-alkanes. This is in agreement with other studies that reported a loss of 22% of the leaf water enrichment for hemicellulose sugars (Helliker and Ehleringer, 2002) and 39 to 62% loss of the leaf water enrichment for *n*-alkanes (Gamarra et al., 2016).

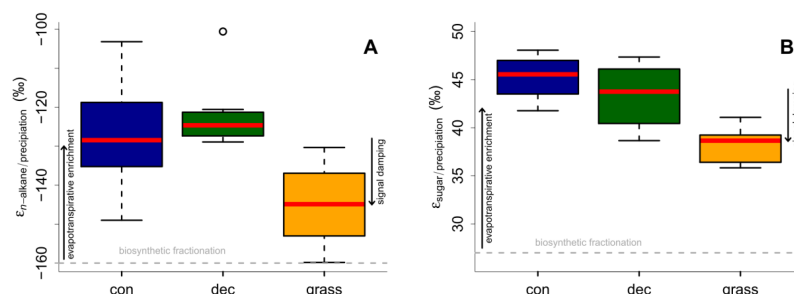


Fig. 7. Apparent fractionation (A) $\epsilon_{n\text{-alkane/precipitation}}$ and (B) $\epsilon_{\text{sugar/precipitation}}$. Biosynthetic fractionation factors according to section 2.4.2. Box plots show median (red line), interquartile range (IQR) with upper (75%) and lower (25%) quartiles, lowest whisker still within 1.5IQR of lower quartile, and highest whisker still within 1.5IQR of upper quartile, dots mark outliers. Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest sites (n=11 and 14 for *n*-alkanes and sugars, respectively); grass = grassland sites (n=4 and 6 for *n*-alkanes and sugars, respectively). The figure conceptually illustrates the effect of biosynthetic fractionation and evapotranspirative enrichment as well as “signal damping”.

3.6 $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ reconstructions

The $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ diagram shown in Fig. 8 graphically illustrates the reconstruction of $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ (colored dots) from $\delta^2\text{H}_{n\text{-alkane}}/\delta^{18}\text{O}_{\text{sugar}}$ (crosses), as well as the reconstruction

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of $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ (black dots). For reconstructing $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$, LELs with an average slope of 2.8 ± 0.1 (Eq. 10) can be generated through every leaf water point and the intercepts of these LELs with the GMWL.

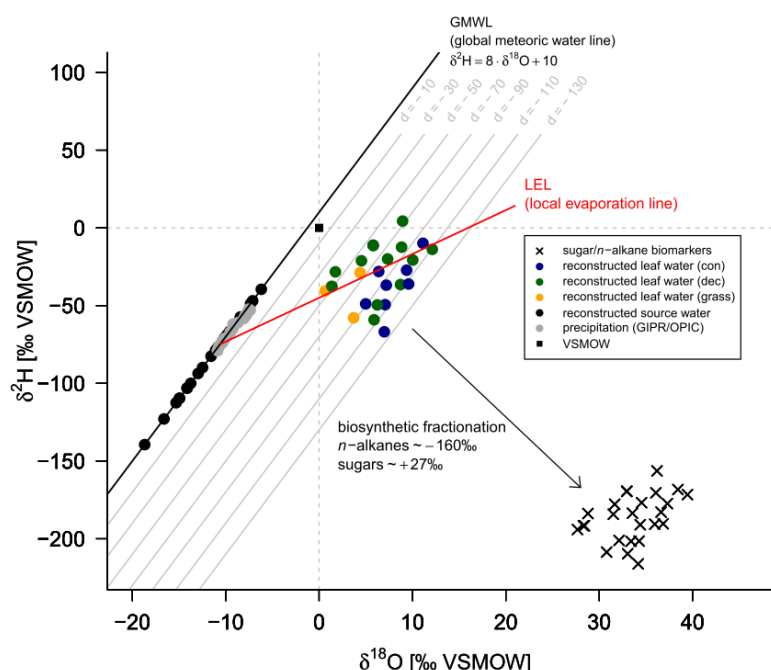


Fig. 8. $\delta^2\text{H}$ vs. $\delta^{18}\text{O}$ diagram illustrating $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$, reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ (according Eqs. 8 and 9) and reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ in comparison to GIPR/OIPC-based $\delta^2\text{H}/\delta^{18}\text{O}_{\text{precipitation}}$. Abbreviations: con = coniferous forest sites ($n=9$); dec = deciduous forest sites ($n=11$); grass = grassland sites ($n=4$).

The reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ results can be compared with the $\delta^2\text{H}/\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ data (Fig. 9). This comparison reveals that the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ approach yields more accurate $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ results than hitherto applied $\delta^2\text{H}_{n\text{-alkane}}$ single isotope approaches. However, the range of the reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ values is clearly larger than in $\delta^2\text{H}/\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ values. $\delta^2\text{H}$ is systematically underestimated by $\sim 21\text{‰} \pm 22$ (Fig. 9B) and $\delta^{18}\text{O}$ by $\sim 2.9\text{‰} \pm 2.8$ (Fig. 9D). The type of vegetation seems to be not particularly relevant ($p\text{-value} = 0.18$ for $\Delta\delta^2\text{H}$ and $p\text{-value} = 0.34$ for $\Delta\delta^{18}\text{O}$). Nevertheless, the systematic offsets tend to be lowest for the deciduous sites ($\Delta\delta^2\text{H}/\Delta\delta^{18}\text{O}$ is closer to zero with $\sim -5\text{‰} \pm 15$ and $\sim -1.1\text{‰} \pm 2.1$), followed by grass sites ($\sim -14\text{‰} \pm 20$ and $\sim -2.1\text{‰} \pm 2.6$). In comparison, the coniferous sites show the largest offsets ($\sim -23\text{‰} \pm 26$ for $\Delta\delta^2\text{H}$ $\sim -3.0\text{‰} \pm 3.3$ for $\Delta\delta^{18}\text{O}$). Differences are, however, not statistically significant. The systematic offset and the large variability might have

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more specific reasons, and we suggest that this is related to the type of vegetation. Deciduous trees produce lots of leaf waxes and sugars (e.g. Prietzel et al., 2013; Zech et al., 2012a), and all biomarkers reflect and record the evapotranspirative enrichment of the leaf water (e.g. Cernusak et al., 2016; Tuthorn et al., 2014). The coupled approach and the leaf water reconstruction based on the *n*-alkane and sugar biomarkers thus works well. However, coniferous trees produce quite low amounts of *n*-alkanes (Diefendorf and Freimuth, 2016; Zech et al., 2012a), while sugar concentrations are as high as in other vascular plants (e.g. Hepp et al., 2016; Prietzel et al., 2013). For the coniferous soil samples this means that the *n*-alkanes stem most likely from the understory whereas the sugars originate from grasses and coniferous needles. When the understory is dominated by grass species then the *n*-alkane biomarkers do not record the full leaf water enrichment signal, whereas the sugars from the needles do. The reconstructed leaf water for the coniferous sites is therefore too negative concerning $\delta^2\text{H}$, and reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ values thus also become too negative (Fig. 8). Concerning the grass sites the following explanation can be found. Correcting for “signal damping” makes the reconstructed leaf water points more positive and shifts them in Fig. 8 up and right. As the “signal damping” is stronger for $\delta^2\text{H}$ than for $\delta^{18}\text{O}$ the corrected leaf water points are now above the uncorrected ones. The corrected leaf water points leads to more positive reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ values for the grass sites.

Vegetation type specific rooting depths could partly cause the overall high variability in reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$. Deep rooting species most likely use the water from deeper soil horizons and/or shallow ground water, which is equal to the (weighted) mean annual precipitation (e.g. Herrmann et al., 1987). Shallow rooting plants take up water from upper soil horizons, which is influenced by seasonal variations in $\delta^2\text{H}/\delta^{18}\text{O}_{\text{precipitation}}$ and by soil water enrichment (Dubbart et al., 2013). Thus, the overall assumption that the source water of the plants reflects the local (weighted) mean precipitation might be not fully valid for all sites. Moreover, a partly contribution of root-derived rather than leaf-derived sugar biomarkers in our topsoil samples is very likely. This does, by contrast, not apply for *n*-alkanes, which are hardly produced in roots (Zech et al., 2012b and the discussion).

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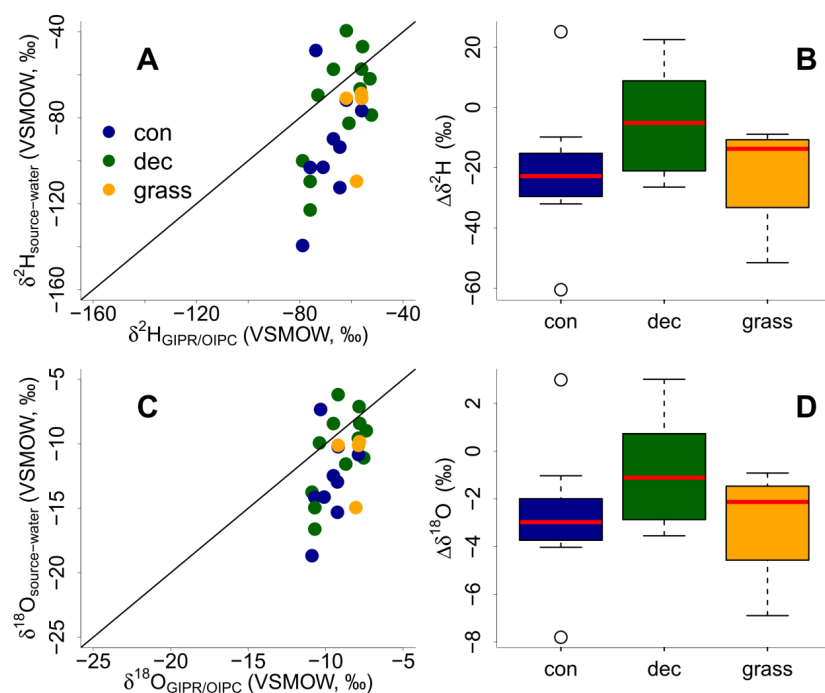


Fig. 9. Correlation of reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ vs. precipitation $\delta^2\text{H}/\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ (A and C). Black lines indicate 1:1 relationship. Differences between reconstructed source water and precipitation ($\Delta\delta^2\text{H}/\delta^{18}\text{O} = \delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}} - \delta^2\text{H}/\delta^{18}\text{O}_{\text{GIPR/OIPC}}$) for the three different vegetation types (B and D). Box plots show median (red line), interquartile range (IQR) with upper (75%) and lower (25%) quartiles, lowest whisker still within 1.5IQR of lower quartile, and highest whisker still within 1.5IQR of upper quartile. Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest sites (n=11); grass = grassland sites (n=4).

Moreover, the high variability within the vegetation types could be caused by variability in ϵ_{bio} of ^2H in *n*-alkanes, as well as ^{18}O in sugars. There is an ongoing discussion about the correct ϵ_{bio} for ^{18}O in hemicellulose sugars (Sternberg, 2014 vs. Zech et al., 2014), and ϵ_{bio} is probably not constant over all vegetation types. This translates into errors concerning leaf water reconstruction and thus for reconstructing $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ values (Eq. 9 and Fig. 8). Likewise, the ϵ_{bio} values reported in the literature for ^2H of *n*-alkanes can be off from -160‰ by tens of permille (Feakins and Sessions, 2010; Tipple et al., 2015; Feakins et al., 2016; Freimuth et al., 2017). The degree to which hydrogen originates from NADPH rather than leaf water is important, because NADPH is more negative (Schmidt et al., 2003). The wide range in biosynthetic ^2H fractionation factors is therefore also related to the carbon and energy metabolism state of plants (Cormier et al., 2018).

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3.7 RH reconstruction

Reconstructed RH_{MDV} ranges from 34 to 74%, while RH_{MDV} from climate station data range from 61 to 78% (Fig. 10A). Biomarker-based values thus systematically underestimate the station data ($\Delta RH_{MDV} = -17\% \pm 12$; Fig. 10B). Yet the offsets are much less for deciduous tree and grass sites ($\Delta RH_{MDV} = -10\% \pm 12$ and $-7\% \pm 9$, respectively). The offsets for the coniferous sites are $-30\% \pm 11$, and significantly larger than for the deciduous and grass sites (p-values < 0.05).

Too low reconstructed RH_{MDV} values for the coniferous sites make sense in view of the previously discussed option that soils contain *n*-alkanes from the understory (which is dominated by grass species), while sugars stem from needles and grasses. As explained earlier already, the “signal damping” leads to too negative reconstructed $\delta^2H_{leaf-water}$ (whereas $\delta^{18}O$ is affected less by the “signal damping”), and too negative $\delta^2H_{leaf-water}$ translates into overestimated d-excess and underestimated RH values. In Fig. 8, a correction for this requires moving the coniferous leaf water data points upwards towards more positive δ^2H values, thus the distance between the leaf water and the source water is reduced.

The underestimation of RH for the deciduous and grass sites could be partly associated with the use of the GMWL as baseline for the coupled $\delta^2H_{n-alkane}-\delta^{18}O_{sugar}$ approach. The deuterium-excess of the LMWLs is generally lower than the +10‰ of the GMWL, while the slopes of the LMWLs are well comparable to the GMWL (Stumpp et al., 2014). In addition, if soil water evaporation occurred before water uptake by the plants, this would lead to an underestimation of biomarker-based RH_{MDV} values. It can be furthermore assumed that plant metabolism is highest during times with direct sunshine and high irradiation, i.e. during noon at sunny days. The relevant RH could therefore be lower than the climate station-derived RH_{MDV} . Indeed, already climate station RH_{MDV} is considerably lower than RH_{MA} and RH_{MV} (Tab. S1).

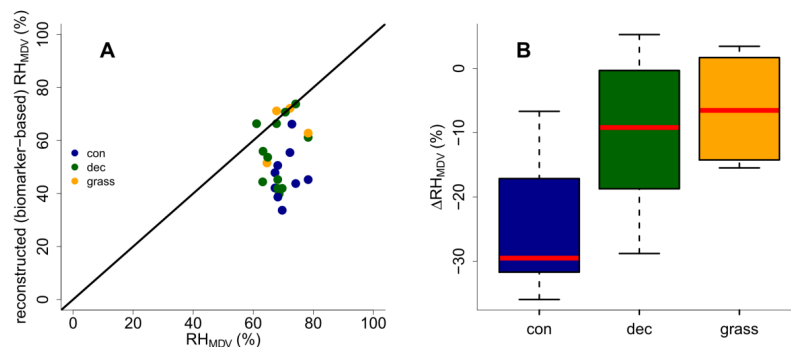


Fig. 10. (A) Comparison of reconstructed (biomarker-based) RH_{MDV} values and climate station RH_{MDV} data. The black line indicates the 1:1 relationship. (B) Differences between reconstructed and climate station RH_{MDV} values ($\Delta RH_{MDV} = \text{reconstructed} - \text{climate station } RH_{MDV}$) for the three different vegetation types along the transect. Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest sites (n=11); grass = grassland sites (n=4).

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The uncertainty of reconstructed RH_{MDV} values are large for all three investigated vegetation types, and again these uncertainties are probably also related to ε_{bio} , which is most likely not constant as assumed for our calculations. Moreover, microclimate variability is underestimated in our approach. As mentioned in sections 2.4.2 and 3.6, in the coupled approach not only the source water of the plants is equated with (weighted) mean annual precipitation, but also an isotopic equilibrium between the source water and the (local) atmospheric water vapour is assumed. However, in areas with distinct seasonality this might be not fully valid. To account for this lack of equilibrium between precipitation and local atmospheric water vapour, apparent ε values can be calculated with data from Jacob and Sonntag, (1991). As shown by Hepp et al. (2018) those values can be used to achieve alternative RH reconstructions based on the coupled $\delta^2H_{n\text{-alkane}}\text{-}\delta^{18}O_{sugar}$ approach. Such calculated RH_{MDV} values are on average 1.5% more negative than the original values. However, this difference in RH is far below the analytical uncertainties of the compound-specific biomarker isotope analysis.

Finally, the integration time of the investigated topsoils has to be discussed. Unfortunately, no ^{14}C dates are available for the soil samples. However, most likely the organic matter has been built up over a longer timescale than the available climate data, which is used for comparison. In combination with vegetation changes/management changes throughout that period, this could surely lead to a less tight relationship of the reconstructions compared to the climate station data. Root input of arabinose and xylose seems to be of minor relevance in our topsoil samples. Otherwise, the reconstructed $\delta^{18}O_{sugar}$ values would be too negative resulting in RH_{MDV} overestimations, which is not observed.

4 Conclusions

We were able to show that

- (i) the vegetation type does not significantly influence the brGDGT concentrations and proxies, yet the coniferous sites tend to have higher brGDGT concentrations, BIT indices and CBT/MBT⁺ ratios, while grass sites tend to be lowest.
- (ii) CBT faithfully records soil pH with a median ΔpH of 0.6 ± 0.6 . The CBT overestimates the real pH particularly at the forest sites.
- (iii) CBT-MBT⁺-derived T_{MA} reflect the climate station-derived T_{MA} values with a median ΔT_{MA} of $0.5^\circ C \pm 2.4$, but again slightly too high reconstruction for the forest sites were observed.
- (iv) differences in the apparent fractionation between the investigated vegetation types are caused by “signal damping”, i.e. the grasses do not see and record the full evaporative enrichment of leaf water.
- (v) the reconstructed $\delta^2H/\delta^{18}O_{source\text{-}water}$ reflects the $\delta^2H/\delta^{18}O_{GIPR/OIPC}$ with a systematic offset for δ^2H of $\sim 21\text{‰} \pm 22$ and for $\delta^{18}O$ of $\sim 2.9\text{‰} \pm 2.8$ (based on overall medians of $\Delta\delta^2H/\delta^{18}O$). This is caused by too negative reconstructions for coniferous and grass sites. For coniferous sites, this can be explained with *n*-alkanes originating from understory grasses, and for the grass sites the “signal damping” more effect δ^2H than $\delta^{18}O$. This leads to too negative reconstructed $\delta^2H_{leaf\text{-}water}$ values and thus to too negative $\delta^2H/\delta^{18}O_{source\text{-}water}$ reconstruction.

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(vi) reconstructed (biomarker-based) RH_{MDV} values tend to underestimate climate station-derived RH_{MDV} values ($\Delta RH_{MDV} = \sim -17\% \pm 12$). For coniferous sites the underestimations are strongest, which can be explained with understory grasses being the main source of *n*-alkanes for the investigated soils under coniferous forests.

Overall, our study highlights the great potential of GDGTs and the coupled $\delta^2H_{n-alkane}-\delta^{18}O_{sugar}$ approach for more quantitative paleoclimate reconstructions. Taking into account effects of different vegetation types improves correlations and reconstructions. This holds particularly true for the coupled $\delta^2H_{n-alkane}-\delta^{18}O_{sugar}$ approach, which is affected by “signal damping” of the grass vegetation. Assuming constant biosynthetic fractionation is likely a considerable source of uncertainty. Climate chamber experiments would be very useful to further evaluate and refine the coupled $\delta^2H_{n-alkane}-\delta^{18}O_{sugar}$ approach, because uncertainties related to microclimate variability can be reduced. Field experiments like ours suffer from the fact that biomarker pools in the sampled topsoils may have been affected by past vegetation and climate changes.

574

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Supplementary figures

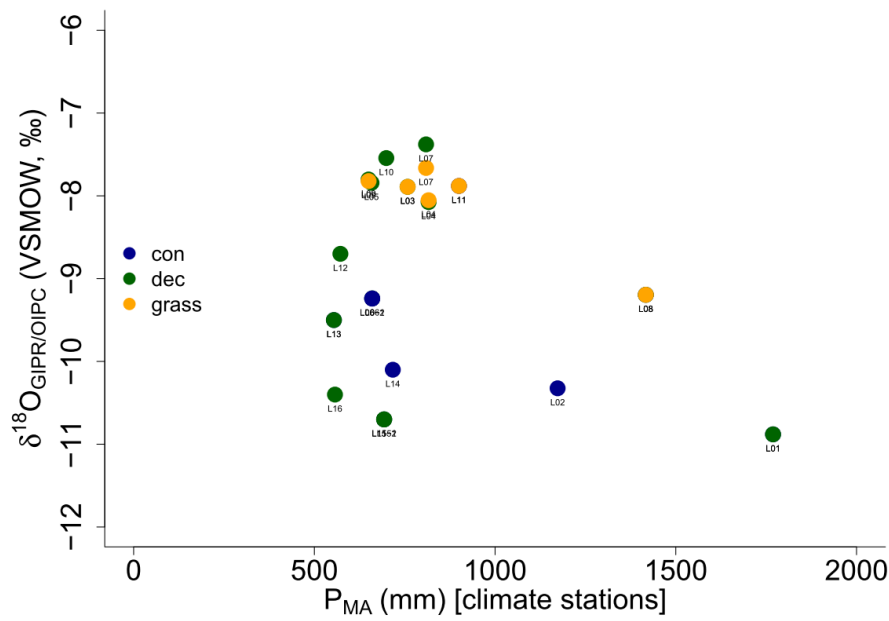
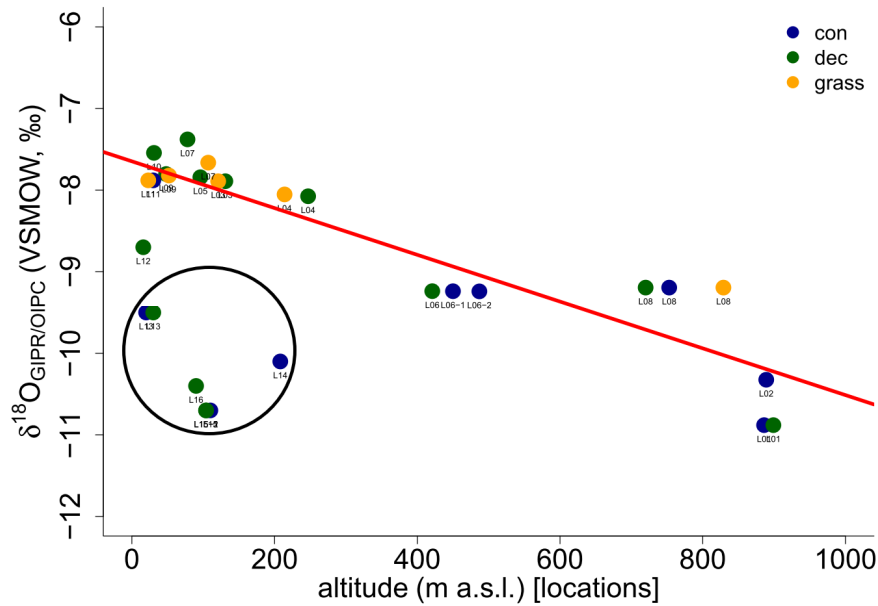
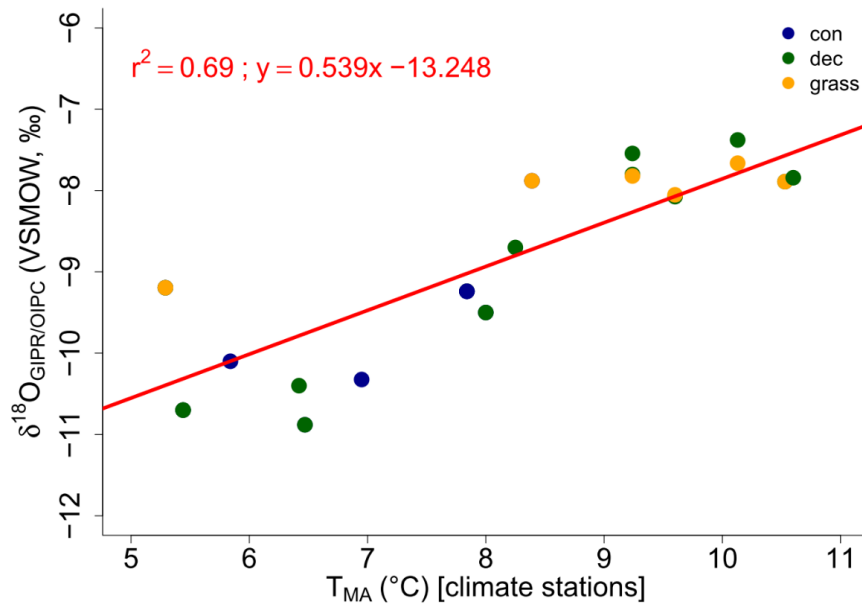


Fig. S1. Comparison between $\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ values vs. P_{MA} for the three different vegetation types along the transect. All data points are marked with the location names. Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest sites (n=11); grass = grassland sites (n=4).



10

11 **Fig. S2.** Comparison between $\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ values vs. location altitudes for the three different
 12 vegetation types along the transect. The red line represents the regression line throughout all
 13 German sites. All data points are marked with the location names. Swedish and Danish sites are
 14 boarded in black. Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest
 15 sites (n=11); grass = grassland sites (n=4).



16 **Fig. S3.** Comparison between $\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ values vs. T_{MA} for the three different vegetation
 17 types along the transect. The red line represents the regression line throughout all sites.
 18 Abbreviations: con = coniferous forest sites (n=9); dec = deciduous forest sites (n=11); grass =
 19 grassland sites (n=4).

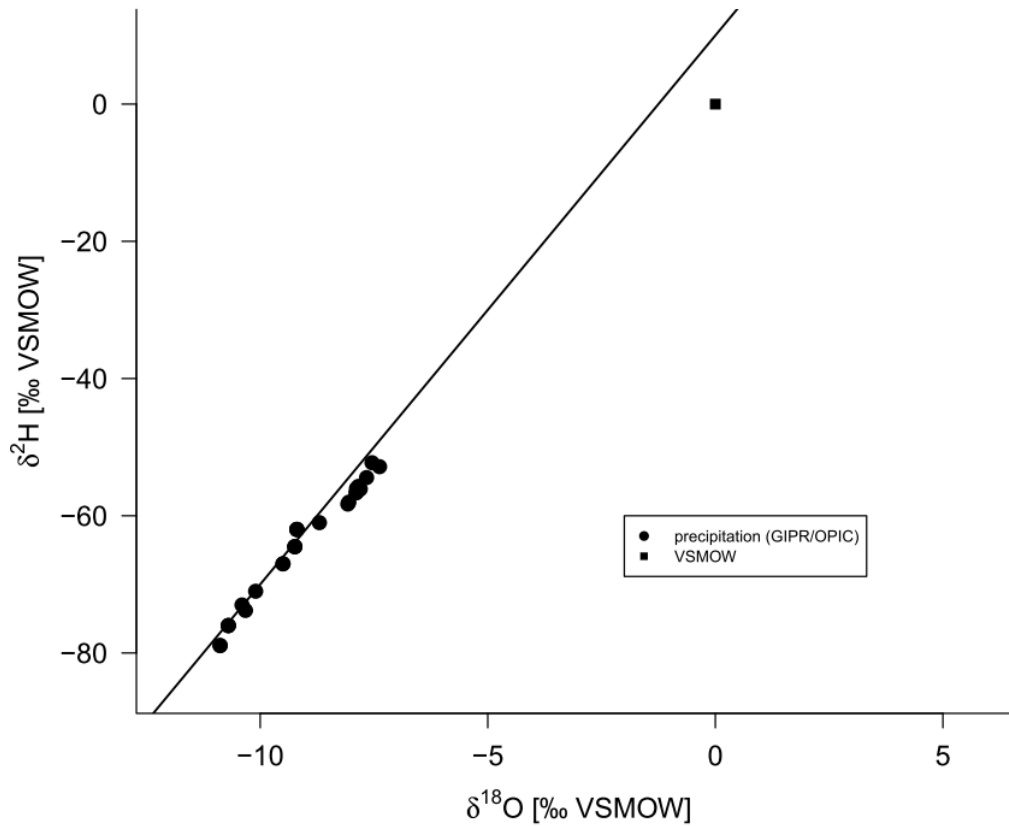


Fig. S4. $\delta^2\text{H}_{\text{GIPR/OIPC}}$ vs. $\delta^{18}\text{O}_{\text{GIPR/OIPC}}$ diagram along the transect. The black line represents the global meteoric water line (GMWL; $\delta^2\text{H} = 8 \times \delta^{18}\text{O} + 10$; Dansgaard, 1964).

Based on the values quoted in the Tabs. S1 and S2, $\delta^{18}\text{O}$ is plotted as functions of the reported environmental parameters (climate station P_{MA} , location altitude and T_{MA} ; Figs. S1 to S3).

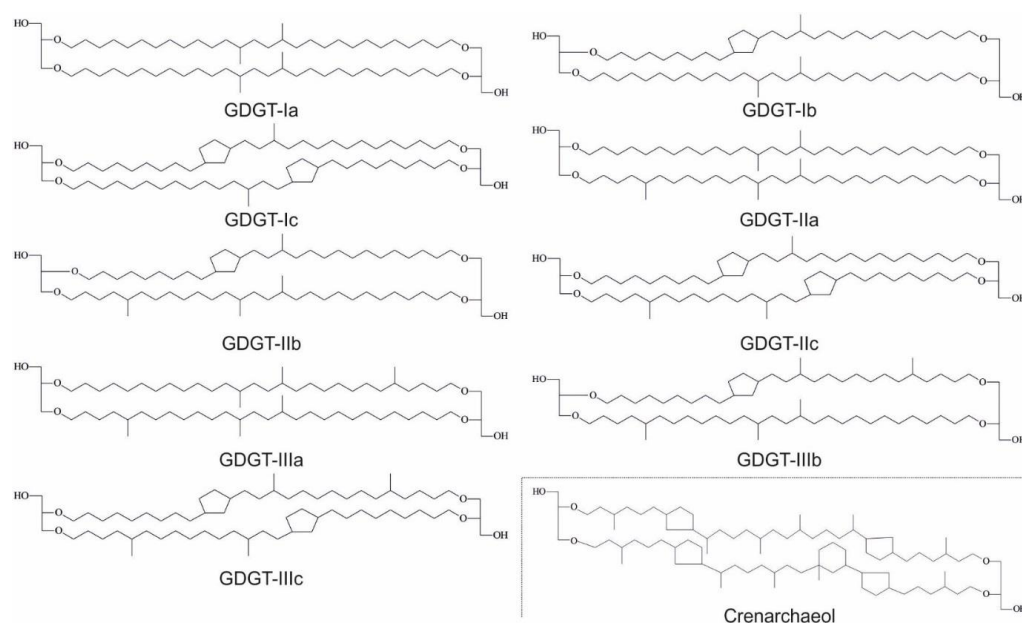
It is worth to note that the five points representing Danish and Swedish sites (L12 to L16) form a separate group in Figs. S2 and S3, with clear more negative $\delta^{18}\text{O}$ values. All other (continental) sites show a regular altitude effect (decreasing $\delta^{18}\text{O}$ values with increasing altitude; red trend in Fig. S3). All Danish and Swedish isotope signatures of precipitation are shifted from the trend line by ca 2 to 2.5‰ towards more negative $\delta^{18}\text{O}$ values. One would rather expect more enriched values due to relative proximity to the sea. It should be noted that those values were derived from OIPC, while the $\delta^{18}\text{O}$ data for the German sites is derived from GNIP/ANIP data (see section 2.2 for more details).

The precipitation $\delta^{18}\text{O}$ shows the expected relationship with T_{MA} (Fig. S4). The slope of this relationship (ca. 0.54‰/°C) is in the range of the slope of δ -T spatial relationship observed at mid latitudes of the northern hemisphere (e.g. Rozanski et al., 1993).

It is apparent from the above Fig. S5 that the data points plot along the GMWL. Only more positive $\delta^{18}\text{O}$ values cluster below the line, indicating most probably some evaporation

38 enrichment effects (partial evaporation of raindrops and/or evaporation effects in the rain
39 gauges).

40



41 **Fig. S5.** Structures of brGDGTs and Crenarchaeol mentioned.

42

43 Literature

44 Dansgaard, W.: Stable isotopes in precipitation, *Tellus*, 16(4), 436–468, doi:10.1111/j.2153-
45 3490.1964.tb00181.x, 1964.

46 Rozanski, K., Araguás-Araguás, L. and Gonfiantini, R.: Isotopic patterns in modern global
47 precipitation, *Climate change in continental isotopic records*, 1–36, 1993.

48 **Supplementary data**49 **Tab. S1.** Location characterization, GIPR and OIPC data.

Location	Vegetation	Characterization	Precipitation $\delta^2\text{H}$ (‰)	Precipitation $\delta^{18}\text{O}$ source (‰)
L01	con	spruce forest, steep hillside	-78.9	GIPR ^{A,B,C,D}
L01	dec	beech forest, close to fir stand	-78.9	GIPR ^{A,B,C,D}
L02	con	fir forest	-73.8	GIPR ^{A,B,C,D}
L03	dec	beeches, oaks, limes, sparse pines	-56.6	GIPR ^{A,B,C,D}
L03	grass	glade, next to farmland and fruit trees (apple, plum)	-56.6	GIPR ^{A,B,C,D}
L04	dec	beech forest, sparse firs and oaks	-58.3	GIPR ^{A,B,C,D}
L04	grass	grassland in the valley, next to beech forest	-58.0	GIPR ^{A,B,C,D}
L05	dec	oak forest, sparse beeches, elms and pines	-55.7	GIPR ^{A,B,C,D}
L06	dec	beech forest, steep hillside	-64.5	GIPR ^{A,B,C,D}
L06-1	con1	sparse pine forest with grass layer	-64.5	GIPR ^{A,B,C,D}
L06-2	con2	sparse larch forest with grass layer	-64.5	GIPR ^{A,B,C,D}
L07	dec	beeches, acers, elms, oaks	-52.8	GIPR ^{A,B,C,D}
L07	grass	heath	-54.4	GIPR ^{A,B,C,D}
L08	con	luxuriant spruce forest	-62.0	GIPR ^{A,B,C,D}
L08	dec	young beech forest at hillside, close to spruce stand	-62.0	GIPR ^{A,B,C,D}
L08	grass	heath, small shrubs, close to spruce stand, initially cleared	-62.0	GIPR ^{A,B,C,D}
L09	dec	birch forest with small oaks, sparse poplars, surrounded by farmland	-56.1	GIPR ^{A,B,C,D}
L09	grass	next to farm track	-56.2	GIPR ^{A,B,C,D}
L10	dec	beech-oak forest	-52.3	GIPR ^{A,B,C,D}
L11	con	spruce forest with larches	-56.0	GIPR ^{A,B,C,D}
L11	grass	cow pasture, sparse oaks	-56.0	GIPR ^{A,B,C,D}
L12	dec	acer forest with poplars, ashes and elder	-61.0	GIPR ^{A,B,C,D}
L13	con	fir forest with swampy underground	-67.0	OIPC ^{E,F,G}
L13	dec	beech forest with sparse acers, birches, loamy underground	-67.0	OIPC ^{E,F,G}
L14	con	spruce-pine forest with moss layer	-71.0	OIPC ^{E,F,G}
L15	con	spruce forest, sparse birches, used as cattle run	-76.0	OIPC ^{E,F,G}
L15-1	dec1	acers, oaks, beeches, sparse firs, on partly pebbly, partly humus-rich floor	-76.0	OIPC ^{E,F,G}
L15-2	dec2	birch- and oak-belt at spruce forest edge, grass layer, also used as cattle run	-76.0	OIPC ^{E,F,G}
L16	dec	oak forest, sparse birches and larches	-73.0	OIPC ^{E,F,G}

^A Stump, C., Klaus, J., Stichler, W., 2014. Analysis of long-term stable isotopic composition in German precipitation. *Journal of Hydrology* 517, 351–361.

^B IAEA/WMO, 2018. Global Network of Isotopes in Precipitation. The GNIP Database, <https://nucleus.iaea.org/wiser>.

^C van Geldern, R., Baier, A., Subert, H.L., Kowol, S., Balk, L., Barth, J.A.C., 2014. (Table S1) Stable isotope composition of precipitation sampled at Erlangen, Germany between 2010 and 2013 for station GeoZentrum located at Erlangen city center, in: In Supplement to: Van Geldern, R. et al. (2014): Pleistocene Paleo-Groundwater as a Pristine Fresh Water Resource in Southern Germany – Evidence from Stable and Radiogenic Isotopes. *Science of the Total Environment*, 496, 107–115, <https://doi.org/10.1016/j.scitotenv.2014.06.061>.

^D Umweltbundesamt GmbH, 2018. Erhebung der Wassergüte in Österreich gemäß Hydrographiegesetz i.d.F. des BGBl. Nr. 252/90 (ültig bis Dezember 2006) bzw. Gewässerzustandsüberwachung in Österreich gemäß Wasserrechtsgesetz, BGBl. I Nr. 123/06, i.d.g.F.: BMLFUW, Sektion IV / Abteilung 3 N, Öffentliche Qualitätsdaten-Abfrage.

^E Bowen, G.J., 2018. The Online Isotopes in Precipitation Calculator, version 3.1, <http://www.waterisotopes.org>.

^F IAEA/WMO, 2015. Global Network of Isotopes in Precipitation. The GNIP Database, <https://nucleus.iaea.org/wiser>.

^G Bowen, G.J., Revenaugh, J., 2003. Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Research* 39, 1–13.

50 **Tab. S2.** Climate station data.

Location	Vegetation	Station ID	Name	Latitude (decimal °)	Longitude (decimal °)	Altitude (m)	Observation begin (YYYYMMDD)	Observation end (YYYYMMDD)	T _{air} (°C)	T _{soil} (°C)	Observation begin (YYYYMMDD)	Observation end (YYYYMMDD)	T _{soil} (°C)	Station ID	Name	Latitude (decimal °)
L01	con	3730	Oberstdorf	47.40	10.28	806	19480101	20171231	6.5 ^A	11.5 ^A	n.n.	n.n.	14.2 ^A	n.n.	n.n.	n.n.
L01	dec	3730	Oberstdorf	47.40	10.28	806	19480101	20171231	6.5 ^A	11.5 ^A	n.n.	n.n.	14.2 ^A	n.n.	n.n.	n.n.
L02	con	2290	Hohenpeißenberg	47.80	11.01	977	19470101	20171231	7.0 ^A	11.4 ^A	n.n.	n.n.	12.7 ^A	n.n.	n.n.	n.n.
L03	dec	2522	Karlsruhe	49.04	8.36	112	19480101	20081102	10.5 ^A	15.3 ^A	n.n.	n.n.	17.8 ^A	n.n.	n.n.	n.n.
L03	grass	2522	Karlsruhe	49.04	8.36	112	19480101	20081102	10.5 ^A	15.3 ^A	n.n.	n.n.	17.8 ^A	n.n.	n.n.	n.n.
L04	dec	3761	Öhringen	49.21	9.52	276	19550101	20171231	9.6 ^A	14.4 ^A	n.n.	n.n.	16.8 ^A	n.n.	n.n.	n.n.
L04	grass	3761	Öhringen	49.21	9.52	276	19550101	20171231	9.6 ^A	14.4 ^A	n.n.	n.n.	16.8 ^A	n.n.	n.n.	n.n.
L05	dec	5906	Mannheim	49.51	8.56	98	19480101	20171231	10.6 ^A	15.4 ^A	n.n.	n.n.	17.9 ^A	n.n.	n.n.	n.n.
L06	dec	3231	Meiningen	50.56	10.38	450	19790101	20171231	7.8 ^A	12.7 ^A	n.n.	n.n.	14.7 ^A	n.n.	n.n.	n.n.
L06-1	con1	3231	Meiningen	50.56	10.38	450	19790101	20171231	7.8 ^A	12.7 ^A	n.n.	n.n.	14.7 ^A	n.n.	n.n.	n.n.
L06-2	con2	3231	Meiningen	50.56	10.38	450	19790101	20171231	7.8 ^A	12.7 ^A	n.n.	n.n.	14.7 ^A	n.n.	n.n.	n.n.
L07	dec	2667	Köln-Bonn	50.86	7.16	92	19600101	20171231	10.1 ^A	14.4 ^A	n.n.	n.n.	16.7 ^A	n.n.	n.n.	n.n.
L07	grass	2667	Köln-Bonn	50.86	7.16	92	19600101	20171231	10.1 ^A	14.4 ^A	n.n.	n.n.	16.7 ^A	n.n.	n.n.	n.n.
L08	con	2483	Kahler Asten	51.18	8.49	839	19510101	20171231	5.3 ^A	9.6 ^A	n.n.	n.n.	10.9 ^A	n.n.	n.n.	n.n.
L08	dec	2483	Kahler Asten	51.18	8.49	839	19510101	20171231	5.3 ^A	9.6 ^A	n.n.	n.n.	10.9 ^A	n.n.	n.n.	n.n.
L08	grass	2483	Kahler Asten	51.18	8.49	839	19510101	20171231	5.3 ^A	9.6 ^A	n.n.	n.n.	10.9 ^A	n.n.	n.n.	n.n.
L09	dec	2014	Hammer	52.46	9.68	55	19490101	20171231	9.2 ^A	13.7 ^A	n.n.	n.n.	15.9 ^A	n.n.	n.n.	n.n.
L09	grass	2014	Hammer	52.46	9.68	55	19490101	20171231	9.2 ^A	13.7 ^A	n.n.	n.n.	15.9 ^A	n.n.	n.n.	n.n.
L10	dec	691	Bremen	53.05	8.80	4	19490101	20171231	9.2 ^A	13.6 ^A	n.n.	n.n.	15.7 ^A	n.n.	n.n.	n.n.
L11	con	4466	Schleswig	54.53	9.55	43	19510101	20171231	8.4 ^A	12.6 ^A	n.n.	n.n.	14.4 ^A	n.n.	n.n.	n.n.
L11	grass	4466	Schleswig	54.53	9.55	43	19510101	20171231	8.4 ^A	12.6 ^A	n.n.	n.n.	14.4 ^A	n.n.	n.n.	n.n.
L12	dec	06120	Odense Lufthavn	55.48	10.33	15	19610101	20001231	8.3 ^C	12.5 ^C	n.n.	n.n.	n.a.	n.n.	n.n.	n.n.
L13	con	30110	Spodsbjerg	55.98	11.85	34	19610101	19901231	8.0 ^C	12.5 ^C	n.n.	n.n.	n.a.	n.n.	n.n.	n.n.
L13	dec	30110	Spodsbjerg	55.98	11.85	34	19610101	19901231	8.0 ^C	12.5 ^C	n.n.	n.n.	n.a.	n.n.	n.n.	n.n.
L14	con	74180	Hagshult Mo	57.29	14.13	169	19430101	20180601	5.8 ^F	10.8 ^F	19490101	20180601	14.5 ^F	n.n.	n.n.	n.n.
L15	con	84580	Snarlunda	58.97	14.90	144/140	19440101	19830901	5.4 ^F	10.8 ^F	19941014	19830831	13.9 ^F	85460	Kettstaka A	58.72
L15-1	dec1	84580	Snarlunda	58.97	14.90	144/140	19440101	19830901	5.4 ^F	10.8 ^F	19941014	19830831	13.9 ^F	85460	Kettstaka A	58.72
L15-2	dec2	84580	Snarlunda	58.97	14.90	144/140	19440101	19830901	5.4 ^F	10.8 ^F	19941014	19830831	13.9 ^F	85460	Kettstaka A	58.72
L16	dec	85330	Motala Kraftverk	58.55	15.08	94	19340101	19901228	6.4 ^F	11.6 ^F	19610101	19851024	14.9 ^F	84310	Karlsborg Mo	58.51

n.n. = not needed/see information further left

n.a. = not available

^A DWD Climate Data Center, 2018a. Historical hourly station observations of 2m air temperature and humidity for Germany, version v006.^B DWD Climate Data Center, 2018b. Historical annual precipitation observations for Germany, version v007.^C Laursen, E.V., Thomsen, R.S., Cappelen, J., 1999. Observed Air Temperature, Humidity, Pressure, Cloud Cover and Weather in Denmark - with Climatological Standard Normals, 1961-90.^D Cappelen, J., 2002. Danish Climatological Normals 1971-2000 - for selected stations.^E Frich, P., Rosenørn, S., Madsen, H., Jensen, J.J., 1997. Observed Precipitation in Denmark, 1961-90.^F Swedish Meteorological and Hydrological Institute, 2018. SMHI Open Data Meteorological Observations, <https://opendata-download-metobs.smhi.se/explore/>.

51 **Tab. S2.** continuation...

Longitude (decimal °)	Altitude (m)	Observation begin (YYYYMMDD)	Observation end (YYYYMMDD)	RH _{low} (%)	RH _{low} (%)	Name	Latitude (decimal °)	Longitude (decimal °)	Altitude (m)	Observation begin (YYYYMMDD)	Observation end (YYYYMMDD)	P _{max} (mm)	Source
n.n.	n.n.	n.n.	n.n.	82 ^A	80 ^A	70 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	1769 ^B	DWD
n.n.	n.n.	n.n.	n.n.	82 ^A	80 ^A	70 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	1769 ^B	DWD
n.n.	n.n.	n.n.	n.n.	78 ^A	77 ^A	73 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	1173 ^B	DWD
n.n.	n.n.	n.n.	n.n.	77 ^A	73 ^A	63 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	758 ^B	DWD
n.n.	n.n.	n.n.	n.n.	77 ^A	73 ^A	63 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	758 ^B	DWD
n.n.	n.n.	n.n.	n.n.	77 ^A	74 ^A	65 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	816 ^B	DWD
n.n.	n.n.	n.n.	n.n.	77 ^A	74 ^A	65 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	816 ^B	DWD
n.n.	n.n.	n.n.	n.n.	75 ^A	71 ^A	61 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	658 ^B	DWD
n.n.	n.n.	n.n.	n.n.	79 ^A	75 ^A	67 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	660 ^B	DWD
n.n.	n.n.	n.n.	n.n.	79 ^A	75 ^A	67 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	660 ^B	DWD
n.n.	n.n.	n.n.	n.n.	79 ^A	75 ^A	67 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	660 ^B	DWD
n.n.	n.n.	n.n.	n.n.	77 ^A	74 ^A	65 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	809 ^B	DWD
n.n.	n.n.	n.n.	n.n.	77 ^A	74 ^A	65 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	809 ^B	DWD
n.n.	n.n.	n.n.	n.n.	87 ^A	84 ^A	78 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	1417 ^B	DWD
n.n.	n.n.	n.n.	n.n.	87 ^A	84 ^A	78 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	1417 ^B	DWD
n.n.	n.n.	n.n.	n.n.	80 ^A	76 ^A	68 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	650 ^B	DWD
n.n.	n.n.	n.n.	n.n.	80 ^A	76 ^A	68 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	650 ^B	DWD
n.n.	n.n.	n.n.	n.n.	80 ^A	77 ^A	69 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	699 ^B	DWD
n.n.	n.n.	n.n.	n.n.	83 ^A	80 ^A	72 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	900 ^B	DWD
n.n.	n.n.	n.n.	n.n.	83 ^A	80 ^A	72 ^A	n.n.	n.n.	n.n.	n.n.	n.n.	900 ^B	DWD
n.n.	n.n.	19800101	19971231	81 ^C	76 ^C	63 ^C	n.n.	n.n.	n.n.	n.n.	n.n.	572 ^E	DMI
n.n.	n.n.	19690101	19921231	84 ^C	80 ^C	74 ^C	n.n.	n.n.	n.n.	n.n.	n.n.	554 ^E	DMI
n.n.	n.n.	19690101	19921231	84 ^C	80 ^C	74 ^C	n.n.	n.n.	n.n.	n.n.	n.n.	554 ^E	DMI
n.n.	n.n.	20130101	20180601	86 ^F	79 ^F	68 ^F	n.n.	n.n.	n.n.	n.n.	n.n.	717 ^F	SMHI
15.03	225	19950801	20180601	82 ^F	75 ^F	68 ^F	Snävlunda D	58.95/58.97/58.97	14.91/14.90/14.90	135/144/140	20150101	693 ^F	SMHI
15.03	225	19950801	20180601	82 ^F	75 ^F	68 ^F	Snävlunda D	58.95/58.97/58.97	14.91/14.90/14.90	135/144/140	20150101	693 ^F	SMHI
15.03	225	19950801	20180601	82 ^F	75 ^F	68 ^F	Snävlunda D	58.95/58.97/58.97	14.91/14.90/14.90	135/144/140	20150101	693 ^F	SMHI
14.51	95	20130101	20180601	83 ^F	78 ^F	71 ^F	Motala	58.56/58.55/58.55	15.02/15.01/15.08	95/95/94	20180501	557 ^F	SMHI

52 **Tab. S3.** GDGT data. Crenarcheol and brGDGTs in µg/g dry weight.

Location	Vegetation	pH (H ₂ O)	Crenarcheol ^a (ng/g dry weight)	IIIa ^a (ng/g dry weight)	IIIb ^a (ng/g dry weight)	IIIc ^a (ng/g dry weight)	IIIA ^a (ng/g dry weight)	IIIB ^a (ng/g dry weight)	IIIC ^a (ng/g dry weight)	Ia ^a (ng/g dry weight)	Ib ^a (ng/g dry weight)	Ic ^a (ng/g dry weight)
L01	con	4.5	2	194	3	0	545	34	1	531	38	7
L01	dec	4.0	1	109	1	0	556	7	3	687	37	10
L02	con	6.5	38	128	9	1	329	81	4	160	86	79
L03	dec	4.3	16	55	0	0	617	17	5	1289	30	9
L03	grass	5.2	12	28	0	0	142	8	1	124	12	2
L04	dec	5.9	13	60	4	1	185	37	3	137	33	6
L04	grass	6.0	208	54	7	3	131	105	8	79	92	27
L05	dec	4.1	15	25	0	0	204	2	1	380	5	1
L06	dec	7.3	16	226	26	1	304	184	6	78	66	5
L06-1	con1	4.5	2	116	0	0	585	18	2	549	21	1
L06-2	con2	6.0	19	332	24	2	695	197	7	295	97	12
L07	dec	3.6	149	67	1	1	506	10	4	677	16	5
L07	grass	4.2	18	19	0	0	141	1	1	183	2	1
L08	con	3.3	29	213	0	0	2265	26	19	3287	32	13
L08	dec	3.6	11	84	0	0	821	12	5	1450	21	8
L08	grass	4.3	0	232	0	0	996	11	2	884	21	6
L09	dec	3.6	64	101	1	0	943	13	5	1513	19	8
L09	grass	4.3	16	26	1	0	169	1	1	275	5	1
L10	dec	3.0	1084	157	33	4	463	68	17	816	23	8
L11	con	3.5	512	76	0	1	353	6	0	406	8	2
L11	grass	5.9	19	89	0	0	579	26	2	714	44	5
L12	dec	4.9	735	450	16	2	2219	418	36	1642	476	142
L13	con	3.2	0	56	0	3	619	0	6	993	13	20
L13	dec	3.7	0	150	0	0	1422	28	16	3165	46	19
L14	con	3.6	0	103	2	0	1180	5	9	2077	17	4
L15	con	3.6	0	207	3	1	2866	48	26	5695	98	35
L15-1	dec1	5.0	7	192	2	0	933	41	4	658	58	22
L15-2	dec2	4.1	5	210	1	0	1896	24	14	2541	41	13
L16	dec	4.3	0	54	0	0	349	5	1	424	9	2

^a structures can be found in Fig. S5^b BIT index was calculated according to Hopmans, E.C., Weijers, J.W.H., Schefuß, E., Herfort, L., Sinnighe Damsté, J.S., Schouten, S., 2004. A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. *Earth and Planetary Science Letters* 224, 107–116.^c MBT, CBT, reconstructed T_{MB} and pHCBT according to Petersen, F., van der Meer, J., Schouten, S., Weijers, J.W.H., Frier, N., Jackson, R.B., Kim, J.H., Sinnighe Damsté, J.S., 2012. Revised calibration of the MBT-CBT paleotemperature proxy based on branched tetraether membrane lipids in surface soils. *Geochimica et Cosmochimica Acta* 96, 215–229.

53 **Tab. S3.** continuation...

brGDGT concentration ($\mu\text{g/g dry weight}$)	BIT	MBT	CBT	reconstructed T_{sea} [$^{\circ}\text{C}$]	pH_{ref}
1.65	1.00	0.35	1.3	4.3	5.37
1.39	1.00	0.53	1.4	9.0	5.05
0.88	0.94	0.37	0.5	9.8	6.98
2.02	0.99	0.66	1.6	12.1	4.74
0.32	0.96	0.43	1.1	7.9	5.69
0.47	0.97	0.38	0.7	8.9	6.59
0.51	0.56	0.40	0.0	13.0	7.84
0.62	0.98	0.63	2.0	9.0	4.01
0.90	0.97	0.17	0.2	5.1	7.54
1.29	1.00	0.44	1.5	6.3	5.04
1.66	0.99	0.25	0.5	5.5	6.86
1.29	0.89	0.54	1.7	8.2	4.63
0.35	0.95	0.53	2.0	5.9	3.90
5.86	1.00	0.57	2.0	7.2	4.00
2.40	1.00	0.62	1.8	9.4	4.26
2.15	1.00	0.42	1.8	3.8	4.39
2.60	0.98	0.59	1.9	8.5	4.19
0.48	0.97	0.59	1.9	8.5	4.23
1.59	0.57	0.55	1.2	11.2	5.63
0.85	0.62	0.49	1.7	6.2	4.50
1.46	0.99	0.52	1.3	9.8	5.40
5.40	0.85	0.42	0.6	10.2	6.65
1.71	1.00	0.60	2.1	7.6	3.78
4.85	1.00	0.67	1.8	11.3	4.37
3.40	1.00	0.62	2.2	7.7	3.64
8.98	1.00	0.65	1.8	10.9	4.42
1.91	1.00	0.39	1.2	6.0	5.52
4.74	1.00	0.55	1.8	7.4	4.29
0.84	1.00	0.52	1.7	6.9	4.46

54 **Tab. S4.** Measured n -alkane $\delta^2\text{H}$ and sugar $\delta^{18}\text{O}$ data along with calculations and reconstruction
 55 results.

Location	Vegetation	n -alkane $\delta^2\text{H}$ (%)	sugar $\delta^{18}\text{O}$ (%)	ϵ_r alkane/precipitation (%)	$\epsilon_{\text{sugar/precipitation}}$ (%)	reconstructed $\delta^2\text{H}_{\text{source-water}}$ (%)	reconstructed $\delta^{18}\text{O}_{\text{source-water}}$ (%)	reconstructed RH_{MDV} (%)
L01	con	-216.2	34.17	-149	45.5	-139	-18.7	34
L01	dec	-190.6	35.95	-121	47.3	-100	-13.8	42
L02	con	-169.4	32.95	-103	43.7	-49	-7.3	66
L03	dec	-176.8	34.54	-127	42.8	-67	-9.6	56
L03	grass	n.a.	29.96	n.a.	38.1	n.a.	n.a.	n.a.
L04	dec	n.a.	35.30	n.a.	43.7	n.a.	n.a.	n.a.
L04	grass	-208.6	30.80	-160	39.2	-110	-14.9	52
L05	dec	-169.6	32.95	-121	41.1	-47	-7.1	66
L06	dec	n.a.	34.30	n.a.	43.9	n.a.	n.a.	n.a.
L06-1	con1	-201.5	34.27	-146	43.9	-113	-15.3	42
L06-2	con2	-191.0	34.39	-135	44.0	-94	-13.0	48
L07	dec	-170.4	36.07	-124	43.8	-62	-9.0	54
L07	grass	n.a.	31.28	n.a.	39.2	n.a.	n.a.	n.a.
L08	con	-168.3	38.42	-113	48.1	-72	-10.2	45
L08	dec	-156.3	36.19	-101	45.8	-40	-6.2	61
L08	grass	-184.2	31.51	-130	41.1	-71	-10.1	63
L09	dec	-177.8	31.66	-129	39.8	-57	-8.4	66
L09	grass	-191.6	28.30	-144	36.4	-69	-9.8	71
L10	dec	-171.6	39.45	-126	47.3	-79	-11.1	40
L11	con	-183.6	33.56	-135	41.8	-77	-10.8	55
L11	grass	-194.1	27.67	-146	35.8	-71	-10.1	72
L12	dec	-177.4	37.30	-124	46.4	-83	-11.6	44
L13	con	-182.9	36.62	-124	46.6	-90	-12.5	44
L13	dec	-183.8	28.79	-125	38.7	-57	-8.4	74
L14	con	-190.3	36.85	-128	47.4	-103	-14.1	39
L15	con	-201.1	32.13	-135	43.3	-103	-14.1	51
L15-1	dec1	-201.6	33.41	-136	44.6	-110	-15.0	45
L15-2	dec2	-209.7	33.05	-145	44.2	-123	-16.6	42
L16	dec	-191.6	28.41	-128	39.2	-69	-9.9	71

n.a. = not available

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journal homepage: www.elsevier.com/locate/quaintLate Quaternary relative humidity changes from Mt. Kilimanjaro, based on a coupled ^2H - ^{18}O biomarker paleohygrometer approach

Johannes Hepp^{a, b, *}, Roland Zech^{c, 1}, Kazimierz Rozanski^d, Mario Tuthorn^{a, b, 2}, Bruno Glaser^b, Markus Greule^e, Frank Keppler^e, Yongsong Huang^c, Wolfgang Zech^f, Michael Zech^{a, b, g, 3}

^a Department of Soil Physics and Chair of Geomorphology, University of Bayreuth, Universitätsstrasse 30, D-95440, Bayreuth, Germany^b Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin-Luther University Halle-Wittenberg, Von-Seckendorff-Platz 3, D-06120, Halle, Germany^c Department of Geological Sciences, Brown University, Providence, RI, 02912, USA^d Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Al. Mickiewicza 30, 30-059, Kraków, Poland^e Institute of Earth Sciences, University of Heidelberg, Im Neuenheimer Feld 236, D-69120, Heidelberg, Germany^f Institute of Soil Science and Geography, University of Bayreuth, 95445 Bayreuth, Universitätsstrasse 30, D-95440, Bayreuth, Germany^g Institute of Geography, Chair of Landscape- and Geoecology, Technical University of Dresden, Helmholtzstrasse 10, D-01062, Dresden, Germany

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ABSTRACT

Our understanding of African paleoclimate/hydrological history is mainly based on lake level and lake sediment studies. It improved during the last decade thanks to emerging stable isotope techniques such as compound-specific deuterium analysis of sedimentary leaf wax biomarkers ($\delta^2\text{H}_{\text{leaf-wax}}$). Here we present the results from a multi-proxy biomarker study carried out on a ~100 ka loess-like paleosol sequence preserved in the Maundi crater at ~2780 m a.s.l. on the southeastern slopes of Mt. Kilimanjaro in equatorial East Africa.

The Maundi stable isotope records established for hemicellulose-derived sugars, lignin- and pectin-derived methoxyl groups, leaf wax-derived fatty acid and *n*-alkane biomarkers ($\delta^{18}\text{O}_{\text{sugars}}$, $\delta^2\text{H}_{\text{methoxyl}}$ groups, $\delta^2\text{H}_{\text{fatty-acids}}$ and $\delta^2\text{H}_{\text{n-alkanes}}$, respectively) reveal similar patterns, but also some distinct differences are obvious. The periods from ~70 to 60 ka, the Last Glacial Maximum (LGM) and the Younger Dryas (YD) are characterized by more positive δ values, whereas during the Holocene, and around 30, 39, and 56 ka BP more negative δ values are determined. The application of a 'coupled $\delta^2\text{H}_{\text{n-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer' approach allows us to derive information about Late Quaternary changes of air relative humidity at the Maundi study site. Reconstructed changes of mean day-time relative humidity (RH_D) are in good agreement with pollen results from the study area. Apart from the overall regional moisture availability, the intensification versus weakening of the trade wind inversion, which affects the diurnal montane atmospheric circulation on the slopes of Mt. Kilimanjaro, is suggested as a local factor which may contribute to the observed variability of RH_D at Maundi study site.

The combined usage of $\delta^2\text{H}_{\text{n-alkanes}}$ and $\delta^{18}\text{O}_{\text{sugars}}$ allowed us to reconstruct $\delta^2\text{H}/\delta^{18}\text{O}$ of source water utilized by plants in the study area, which is directly linked to local precipitation. The results of this reconstruction caution against a straightforward interpretation of $\delta^2\text{H}_{\text{leaf-wax}}$ and $\delta^{18}\text{O}_{\text{sugars}}$ records as proxies for isotopic composition of local precipitation because variable and primarily RH-dependent isotopic evaporative enrichment of leaf water can mask changes of $\delta^2\text{H}_{\text{prec}}/\delta^{18}\text{O}_{\text{prec}}$ in the past. The biomarker-based $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ records derived for the Maundi site revealed a discernible link with the reconstructed RH_D record; lower RH_D values were generally observed during periods characterized by more negative $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ values, indicating a reverse relationship with the expected precipitation amount. This indicates that the empirical relationship between amount of rainfall and its

* Corresponding author. Present address: Chair of Geomorphology, University of Bayreuth, Universitätsstrasse 30, D-95440, Bayreuth, Germany.

E-mail address: johannes-hepp@gmx.de (J. Hepp).¹ Present address: Institute of Geography, Chair of Physical Geography, Friedrich-Schiller University of Jena, Löbdergraben 32, D-07743 Jena, Germany.² Present address: Thermo Fisher Scientific, Bremen, Germany.³ Present address: Institute of Geography, Chair of Landscape- and Geoecology, Technical University of Dresden, Helmholtzstrasse 10, D-01062 Dresden, Germany.

isotopic composition, observed nowadays on monthly timescale in the East African region, might not be valid on millennial time scale.

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1. Introduction

East Africa and its Late Quaternary climate and vegetation history has received much attention during the last decades. Evidence for dramatic environmental and hydrological changes come from various types of archives, such as ice cores (Thompson et al., 2002) and glacial deposits (Mark and Osmaston, 2008; Shanahan and Zreda, 2000), lake sediments (e.g. Berke et al., 2012; Cockerton et al., 2015; Gasse, 2000; Gasse et al., 2008; Scholz et al., 2007; Street-Perrott et al., 2004; Trauth et al., 2003; Verschuren et al., 2009) and marine sediments (e.g. Schefuß et al., 2011; Tierney and deMenocal, 2013). The equatorial and northern East Africa, as well as the east Saharan region, experienced multiple lake level high-stands and humid conditions, especially during the Early Holocene (African Humid Period - AHP). The East African monsoon is responsible for rainy periods in East Africa and is controlled by low-latitude insolation changes occurring on orbital timescales. However, the forcing of the East African monsoon on millennial timescales is still a matter of debate. Evidence for precession forcing, including half-precession effects, have been presented in numerous studies (e.g. Trauth et al., 2003; Verschuren et al., 2009). However, many influencing factors and controlling mechanisms on East African paleoclimate are not yet fully understood. This concerns the teleconnection with high-latitude boundary conditions, for instance during the Younger Dryas period, the possible influence of the Indian Ocean Dipole (IOD) and the El Niño Southern Oscillation (ENSO) phenomena, and the possible influence of a migrating Congo air boundary (Abram et al., 2007; Castañeda et al., 2007; Konecky et al., 2011; Schefuß et al., 2011; Stager et al., 2011; Tierney et al., 2008, 2011). The multitude of possible controls of East African climate in the past stimulate the ongoing research efforts addressing exact timing, abruptness and spatial/temporal variability of East African monsoon precipitation.

During the last decade, the hydrogen isotopic composition of sedimentary leaf waxes ($\delta^2\text{H}_{\text{leaf-wax}}$) became a widely used proxy that was also explored in East African paleoclimate/hydrological archives. There are two major assumptions underlying most interpretations of $\delta^2\text{H}_{\text{leaf-wax}}$ records originating from this region. First, $\delta^2\text{H}$ values of leaf waxes extracted from lake sediments reflect the isotopic composition of paleoprecipitation ($\delta^2\text{H}_{\text{prec}}$) (e.g. Konecky et al., 2011; Tierney et al., 2010, 2011). Second, $\delta^2\text{H}_{\text{leaf-wax}}$ records retrieved from sedimentary archives can be interpreted in terms of an 'amount effect', as inferred from modern precipitation in the tropics (e.g. Schefuß et al., 2005, 2011; Tierney et al., 2008; Tierney and deMenocal, 2013).

However, the first assumption may not be as robust as previously thought. For instance, the ^2H content of leaf wax-derived *n*-alkane biomarkers, studied in a modern topsoil climate transect along the southern slopes of Mt. Kilimanjaro, does not follow the expected 'altitude effect' for $\delta^2\text{H}$ of local precipitation (Zech et al., 2015). The *n*-alkanes were rather found to reflect the isotopic composition of leaf water ($\delta^2\text{H}_{\text{leaf-water}}$), as it was previously suggested by Kahmen et al. (2013). Given that ^2H -enrichment of leaf water strongly depends on relative air humidity (Farquhar et al., 2007; Flanagan et al., 1991; Roden et al., 2000), large changes of this parameter may thus mask climatically-driven fluctuations of $\delta^2\text{H}_{\text{prec}}$.

The second assumption is based on the observation that for

present-day climate monthly means of $\delta^2\text{H}_{\text{prec}}$ ($\delta^{18}\text{O}_{\text{prec}}$) values in the tropics are inversely correlated with the precipitation amount collected at a given site (e.g. Rozanski et al., 1993). This is also true for East Africa (Rozanski et al., 1996). However, on an inter-annual basis, which is the relevant timescale for (paleo-)climatic considerations, such correlation is very poor or not-existent, at least for the East African region (Rozanski et al., 1996), and validation of a long-term 'amount effect' is in fact lacking for this area. Alternatively, Konecky et al. (2011) suggested that moisture source and transport history dominated the $\delta^2\text{H}_{\text{leaf-wax}}$ record at Lake Malawi, whereas rainfall amount played a secondary role.

In order to overcome ambiguities associated with the interpretations of $\delta^2\text{H}_{\text{leaf-wax}}$ records, Zech et al. (2013) suggested a coupled $\delta^2\text{H}_{\text{n-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ biomarker approach, where $\delta^{18}\text{O}_{\text{sugar}}$ is determined by compound-specific $\delta^{18}\text{O}$ -analyses of the hemicellulose-derived sugar biomarkers, such as arabinose, fucose, xylose and rhamnose (Zech and Glaser, 2009). This coupled approach opens up new possibilities: (i) in combination with known biosynthetic fractionation factors (ϵ_{bio}) it enables the reconstruction of the isotopic composition of leaf water [$\delta^2\text{H}_{\text{leaf-water}} = \delta^2\text{H}_{\text{leaf-wax}} - \epsilon_{\text{bio}}$ (*n*-alkanes); $\delta^{18}\text{O}_{\text{leaf-water}} = \delta^{18}\text{O}_{\text{sugars}} - \epsilon_{\text{bio}}$ (sugars)]. (ii) The evapotranspirative ^2H and ^{18}O enrichment of leaf water – characterized by the deuterium-excess of leaf water – can be used to quantify relative humidity of the local atmosphere for the periods when stomata are open and the transpiration process is in operation. Relative air humidity appears to be a decisive factor controlling the extent of this isotope enrichment. Finally, (iii) the intersect of the local leaf water evaporation line (LLEL) with the local meteoric water line (LMWL) can be used to reconstruct $\delta^2\text{H}/\delta^{18}\text{O}$ source water values more robustly than previously done, based on $\delta^2\text{H}_{\text{leaf-wax}}$ records alone. Recently, Tuthorn et al. (2015) validated this coupled $\delta^2\text{H}-\delta^{18}\text{O}$ biomarker approach by applying it to an Argentinean climate topsoil transect. Their findings corroborate that the 'coupled $\delta^2\text{H}_{\text{n-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer' is a promising proxy for reconstructing day-time relative humidity of local atmosphere (RH_D).

The aim of this study was (i) to establish a multi-proxy stable isotope biomarker record spanning the last ~ 100 ka by investigating a loess-like paleosol sequence from the Maundi crater situated on the southeastern slopes of Mt. Kilimanjaro, equatorial East Africa, (ii) to compare the Maundi $\delta^2\text{H}_{\text{n-alkane}}$ record with the $\delta^2\text{H}$ records of fatty acids and lignin-/pectin-derived methoxyl groups ($\delta^2\text{H}_{\text{fatty-acid}}$ and $\delta^2\text{H}_{\text{methoxyl}}$, respectively) as well as with published $\delta^2\text{H}$ biomarker records from East African lakes, (iii) to reconstruct the past history of the RH_D at the Maundi study site using the 'coupled $\delta^2\text{H}_{\text{n-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer', and (iv) to reconstruct and interpret the $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source water}}$ record for the Maundi loess-like paleosol sequence in terms of paleoclimate.

2. Materials and methods

2.1. Study area – the Maundi crater

A detailed description of the study area, as well as an age-depth model of the Maundi loess-like paleosol sequence, were previously presented by Schüller et al. (2012). In brief, Maundi is an ancient volcanic crater of ~60 m diameter and 20–30 m depth that is located on the southeastern slopes of Mt. Kilimanjaro at ~2780 m

a.s.l. (Fig. 1; 3°10'27.5"S, 37°31'05.8"E). The surrounding vegetation represents the transition from closed forest (upper montane forest) to open *Erica* bush (subalpine heathland) (Fig. 1b). The bottom of the crater is occupied by a seasonal swamp, which only holds standing water during the rainy season (Schüler et al., 2012). There are two rainy seasons in the area caused by the seasonal migration of the ITCZ (Fig. 1a); the long rainy season from March to May with subsequently prevailing southeasterly trade winds and the short rainy season from November to December with subsequently prevailing northeasterly trade winds. Apart from seasonal climate variability, pronounced diurnal atmospheric circulation changes are observed along the slopes of Mt. Kilimanjaro (Appelhans et al., 2015; Duane et al., 2008; Pepin et al., 2010). Strong upslope moisture transport occurs during the day, whereas downslope transport and drying occurs at night. The study site is located above the RH maxima of the montane zone, as depicted by Fig. 1c. Mean annual precipitation at Maundi is ~1800 mm and mean annual temperature is about 9 °C (Hemp, 2006b). Mean day-time temperature from a close-by meteorological field station is slightly higher at ~14 °C (cf. Appelhans et al., 2015).

During the Late Quaternary, the Maundi crater served as trap for aeolian and colluvial sediments. In July 2007, samples were taken from a 240 cm deep soil pit and additionally from further down to 646 cm depth using a piston corer. While Schüler et al. (2012) established their age-depth model by adapting a linear trend line through all 11 available calibrated AMS radiocarbon ages, we chose

a linear interpolation between each individual ^{14}C data for the upper 3 m of the sequence and a linear extrapolation for the lower part of the sequence (Fig. 2). The extrapolation suggests that the lowermost part of the core at 6.46 m depth may be as old as ~100 ka BP. Pollen, total organic carbon (TOC) and glycerol dialkyl glycerol tetraether (GDGT) biomarker results were published previously by Schüler et al. (2012) and Zech et al. (2012). The position of sugar, methoxyl and leaf wax (fatty acids and *n*-alkanes) samples are shown in Fig. 2.

2.2. Biomarker and compound-specific $\delta^{18}\text{O}/\delta^2\text{H}$ analyses

A total of 38 samples were prepared for $\delta^{18}\text{O}$ analyses of hemicellulose-derived sugar biomarkers according to Zech and Glaser (2009) at the Department of Soil Physics and the Chair of Geomorphology at the University of Bayreuth. In brief, the hemicelluloses were hydrolytically extracted with 4 M trifluoroacetic acid (TFA) (Amelung et al., 1996); the extracted sugars were cleaned using XAD-7 and Dowex 50WX8 columns; the purified sugars were freeze-dried and afterwards derivatized by adding methylboronic acid (MBA; 4 mg in 400 μl pyridine) and heating for 1 h at 60 °C. The compound-specific $\delta^{18}\text{O}$ measurements were performed at the Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin-Luther University Halle-Wittenberg, using a Trace GC 2000 gas chromatograph (GC; Thermo Fisher Scientific, Bremen, Germany) coupled to a Delta V Advantage isotope ratio mass

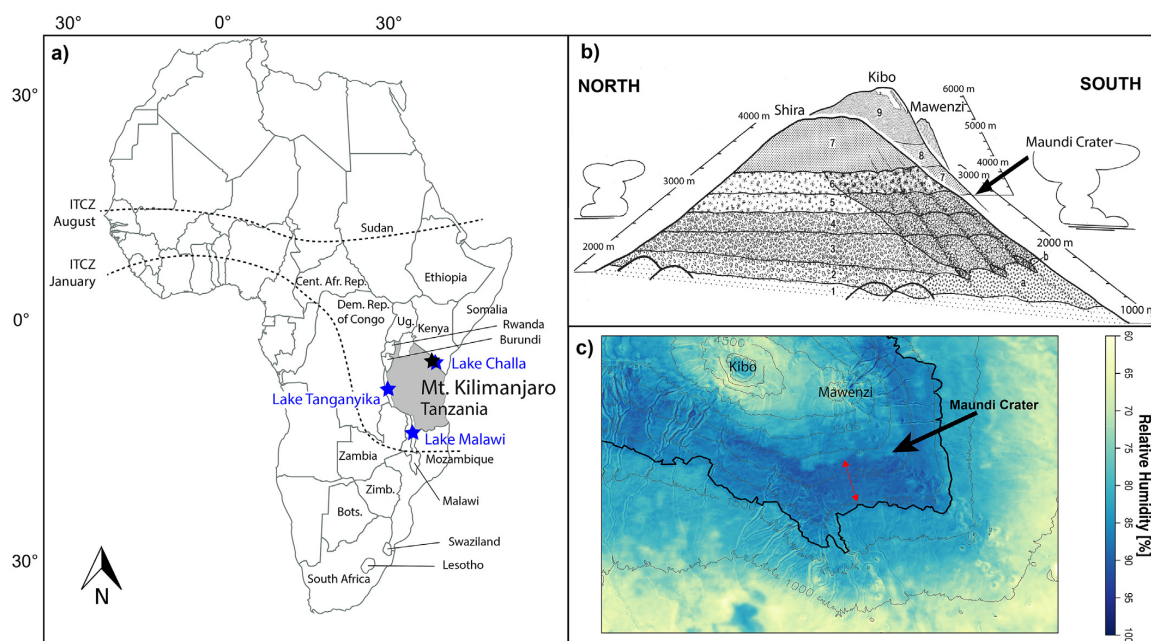


Fig. 1. a) Overview map of Africa depicting the study area Mt. Kilimanjaro, Tanzania. The black star shows the location of Maundi crater. Blue stars indicate locations of Lake Malawi, Lake Tanganyika, and Lake Challa. The dashed lines represent approximate positions of the ITCZ during August and January, respectively. b) North-south profile of the western slope of Mt. Kilimanjaro, showing the three volcanic cones Shira, Kibo and Mawenzi as well as the main altitudinal zones, vegetation types and the Maundi crater. 1: colline (savanna) zone; 2: submontane zone with *Croton-Calodendrum* forest; 3: lower montane zone with *Cassipourea* forests on the northern slope and *Agauria-Syzygium-Ocotea* forests on the southern slope; 4: middle montane zone with *Cassipourea* forests on the northern slope and *Ocotea* forests on the southern slope; 5: upper montane zone with *Juniperus* forests on the northern slope and *Podocarpus-Ocotea* forests on the southern slope; 6: subalpine zone with *Juniperus* forests on the northern slope and *Podocarpus* forests on the southern slope; 7: subalpine zone with heathlands (*Erica* bush); 8: lower alpine zone with *Helichrysum* cushion vegetation; 9: upper alpine and nival zone, mainly bare of vegetation (modified according to Hemp, 2006a). c) Relative humidity map illustrating the characteristic altitudinal hillside RH gradients and the RH maxima in the montane zone (red double arrow) (Appelhans et al., 2015; modified). Subfigures a) and b) reprinted and modified according to Schüler et al. (2012). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

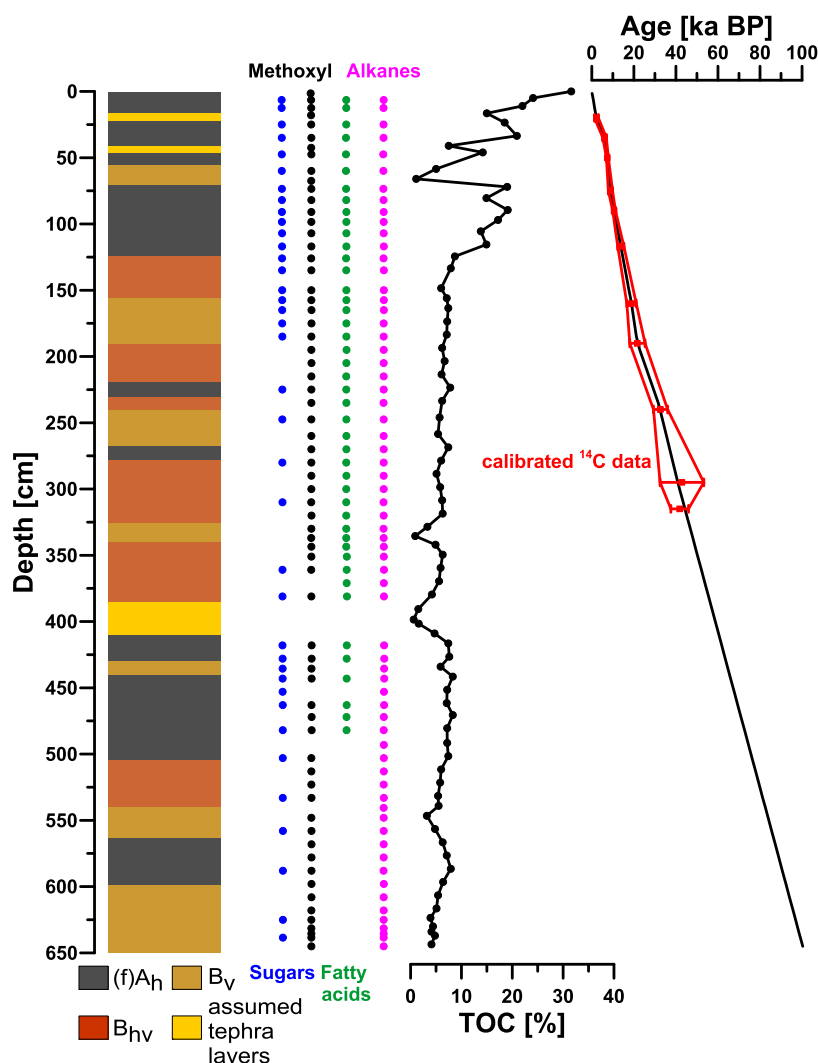


Fig. 2. Left: Stratigraphy of the Maundi loess-like paleosol sequence (modified according to Zech et al., 2012). (f)Ah: (fossil) dark soil horizons; B_v: weathered, brown soil horizons; B_{hv}: weathered, dark brown soil horizons; assumed tephra layers: yellowish smeary sandy silt (presumably strongly weathered tephra layers); and position of sugar (blue dots), methoxyl (black dots) and leaf wax (fatty acids as green dots and n-alkanes as magenta dots) samples. Middle: Total organic carbon (TOC) depth profile. Right: Revised age-depth model (black line) based on 11 calibrated AMS radiocarbon data (modified according to Schüller et al., 2012). Red squares display the calibrated ¹⁴C data with associated measurement uncertainties and uncertainty band (thin red lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

spectrometer (IRMS; Thermo Fisher Scientific, Bremen, Germany) via an ¹⁸O-pyrolysis reactor and a GC/TC III interface (Thermo Fisher Scientific, Bremen, Germany). While arabinose, fucose and xylose yielded peak areas that were high enough for robust peak integrations in the chromatograms, rhamnose was excluded from further data evaluation due to too low peak areas. Mean standard errors for triplicate measurements of all 38 samples are 0.90‰, 1.46‰ and 0.97‰ for arabinose, fucose and xylose, respectively. The three sugars yielded very similar results with correlation coefficients ranging from 0.6 to 0.8 ($p < 0.001$; $n = 38$). In the following, we use the weighted mean $\delta^{18}\text{O}$ values of arabinose, fucose and xylose and refer to as $\delta^{18}\text{O}_{\text{sugars}}$, relative to Vienna

Standard Mean Ocean Water (VSMOW).

Laboratory work for the leaf wax analyses on 74 samples and compound-specific $\delta^2\text{H}$ measurements followed standard procedures at the Department of Geological Sciences, Brown University. In brief, free lipids were extracted using accelerated solvent extraction (Dionex ASE 200) with dichloromethane (DCM) and methanol (MeOH, 9:1). Lipids were separated over pipette columns filled with aminopropyl silica gel (Supelco, 45 μm). n-Alkanes were eluted with hexane, polar lipids with DCM and MeOH (1:1), and fatty acids with acetic acid in diethyl ether (1:19). The fatty acids were methylated using 5% acetyl chloride in methanol of a known isotopic composition, yielding the corresponding fatty acid methyl

esters (FAMES). These were recovered by liquid-liquid extraction using hexane and further purified over silica columns. Quantification of the leaf wax-derived long-chain *n*-alkanes and *n*-fatty acids was done on a HP 6890 GC coupled to a flame ionization detector (Agilent, Santa Clara, CA, USA). Compound-specific $\delta^2\text{H}$ measurements of the *n*-alkanes *n*-C₂₉ and *n*-C₃₁ and the *n*-fatty acids *n*-C₂₆ and *n*-C₂₈ (dominant peaks/compounds and considered to derive from terrestrial higher plants) were performed on a GC-Pyrolysis-IRMS system consisting of HP 6890 GC coupled to a Delta^{PLUS}XL (Thermo-Quest Finnigan, Bremen, Germany). Mean standard errors for triplicate measurements of all 74 *n*-alkane samples were 0.9‰ and 1.1‰ for *n*-C₂₉ and *n*-C₃₁, respectively, and 0.9‰, each, for the fatty acids *n*-C₂₆ and *n*-C₂₈ measured in triplicate for 45 samples. During the course of the measurements the H_3^+ factor stayed constant. The $\delta^2\text{H}$ results of the *n*-alkanes *n*-C₂₉ and *n*-C₃₁ correlate with $R = 0.6$ ($p < 0.001$); their weighted mean values are reported in the following as $\delta^2\text{H}_{n\text{-alkanes}}$, relative to VSMOW. The $\delta^2\text{H}$ results of the fatty acids *n*-C₂₆ and *n*-C₂₈ correlate with $R = 0.8$ ($p < 0.001$); their mean values are reported as $\delta^2\text{H}_{\text{fatty-acids}}$, corrected for the methyl group added during methylation and also relative to VSMOW.

Although methoxyl groups are not specific compounds *sensu stricto*, they originate mostly from lignin and/or pectin. Given that their $\delta^2\text{H}$ values ($\delta^2\text{H}_{\text{methoxyl}}$) were found to reflect $\delta^2\text{H}_{\text{prec}}$, $\delta^2\text{H}_{\text{methoxyl}}$ was recently suggested as a paleoclimate proxy (Anhäuser et al., 2014; Keppeler et al., 2007). The respective analysis was described previously by Greule et al. (2008). In brief, methoxyl groups are converted to gaseous methyl iodide (CH_3I) by the addition of hydroiodic acid (HI) and heating of the samples to 130 °C for 30 min. The $\delta^2\text{H}_{\text{methoxyl}}$ measurements for the Maundi samples were performed using an HP 6890N GC (Agilent, Santa Clara, CA, USA) equipped with an A200S auto-sampler (CTC Analytics, Zwingen, Switzerland), coupled to a Delta^{PLUS}XL IRMS (Thermo-Quest Finnigan, Bremen, Germany) via a pyrolysis reactor and a GC Combustion III interface (Thermo-Quest Finnigan, Bremen, Germany).

3. The coupled $\delta^2\text{H}$ - $\delta^{18}\text{O}$ biomarker paleohygrometer and reconstruction of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of source water

The coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach was previously described in detail by Zech et al. (2013) and Tuthorn et al. (2015). Leaf water undergoes evaporation through stomata openings. This process is associated with equilibrium and kinetic isotope effects, which causes isotopic enrichment of leaf water (Dongmann et al., 1974). Due to its small dimensions, the leaf water reservoir at evaporation sites quickly reaches isotope steady-state in which the isotopic composition of water vapor leaving the leaf surface is identical to the isotopic composition of the 'source' water pumped by plants from the ground in the course of the transpiration process (Flanagan et al., 1991; Roden and Ehleringer, 1999). In most cases the water used up by plants is directly linked through soil water and shallow groundwater to the local precipitation.

If the isotope steady-state of the leaf water reservoir is assumed, its ^2H and ^{18}O isotope composition can be then calculated using a 'terminal lake analogue' (cf. Zech et al., 2013 adopted from Gat and Bowser, 1991):

$$\delta_{\text{leaf-water}} \cong \delta_{\text{source-water}} + (1 - h_N)\epsilon^* + \Delta\epsilon \quad (1)$$

where $\delta_{\text{leaf-water}}$ and $\delta_{\text{source-water}}$ is the isotopic composition of leaf water and source (transpired) water, respectively, expressed in (‰), h_N is the relative humidity of the local atmosphere, normalized to the leaf water temperature; $\epsilon^* = (1 - 1/\alpha_{LV})10^3$ is the equilibrium isotope enrichment where α_{LV} stands for equilibrium isotope

fractionation between the liquid and gaseous phase (‰), and $\Delta\epsilon$ is the kinetic isotope enrichment [$\Delta^{18}\epsilon = C_k^{18}(1 - h_N)$; $\Delta^2\epsilon = C_k^2(1 - h_N)$] where C_k^{18} , C_k^2 stand for kinetic enrichment parameters, for ^{18}O and ^2H , respectively.

When $\delta^2\text{H}_{\text{leaf-water}}$ and $\delta^{18}\text{O}_{\text{leaf-water}}$ values are known, the d-excess parameter for the leaf water reservoir ($d_{\text{leaf-water}}$) undergoing evaporation, defined as $d = \delta^2\text{H} - 8 \cdot \delta^{18}\text{O}$, can be calculated using Eq. (1):

$$d_{\text{leaf-water}} = d_{\text{source-water}} + (1 - h_N)(\epsilon_2^* - 8 \cdot \epsilon_{18}^* + C_k^2 - 8 \cdot C_k^{18}) \quad (2)$$

where $d_{\text{source-water}}$ is the d-excess of the source water. It is apparent from Eq. (2) that the d-excess of leaf water is primarily controlled by the relative humidity of the local atmosphere when stomata are open and the transpiration process is in operation.

If the d-excess of leaf water is quantified through $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurements of relevant biomarkers and the d-excess of source water is known or can be assumed, the relative humidity of the local atmosphere can be estimated from Eq. (2):

$$h_N = 1 - \frac{\Delta d}{\epsilon_2^* - 8 \cdot \epsilon_{18}^* + C_k^2 - 8 \cdot C_k^{18}} \quad (3)$$

where $\Delta d = d_{\text{leaf-water}} - d_{\text{source-water}}$ stands for the difference of the d-excess values of the leaf water and the source water. Although kinetic enrichment parameters, C_k , can vary widely depending on the aerodynamic conditions characterizing a given evaporation process, maximum values of those parameters (25.1‰ and 28.5‰ for C_k^2 and C_k^{18} , respectively; Merlivat, 1978) seem to be most suitable for leaf water evaporation (see Zech et al. (2013) for further discussion). Equilibrium isotope enrichments, ϵ_2^* and ϵ_{18}^* , as a function of temperature can be calculated using empirical equations (Horita and Wesolowski, 1994; Majoube, 1971). It is to be noted here that evaporation of leaf water takes place when stomata are open i.e. during photosynthetic activity of a plant. While the biomarkers are synthesized during day-time in C_3 plants, which are of relevance here, the relative humidity defined by Eq. (3) (h_N) is in fact a proxy for day-time relative humidity (RH_p) of the local atmosphere seen by transpiring plants.

Equation (3) provides a useful tool to establish relative humidity records from sedimentary archives provided that: (i) ^2H and ^{18}O isotope composition of leaf water reservoir can be reconstructed using the measured δ values of *n*-alkanes and sugars and respective biosynthetic fractionation factors (ϵ_{bio}), and (ii) the d-excess of local source water (precipitation) can be evaluated. As far as point (i) is considered, we are aware that the biosynthetic fractionation factors for biosynthesis of *n*-alkanes and sugars in plants are strictly speaking not constant, however, we consider the respective ϵ_{bio} values not to be substantially variable (Sternberg, 2014 vs. Zech et al., 2014a). We therefore apply an ϵ_{bio} value of -160‰ for reconstructing $\delta^2\text{H}_{\text{leaf-water}}$ from measured $\delta^2\text{H}$ of *n*-alkanes (Sachse et al., 2006; Sessions et al., 1999) and an ϵ_{bio} value of $+27\text{‰}$ for reconstructing $\delta^{18}\text{O}_{\text{leaf-water}}$ from measured $\delta^{18}\text{O}$ of sugars (Cernusak et al., 2003; Gessler et al., 2009; Schmidt et al., 2001; Sternberg et al., 1986; Yakir and DeNiro, 1990). Note that interspecies variation in the fractionation between leaf water and leaf wax $\delta^2\text{H}$ (Kahmen et al., 2013), as well as other potentially biological processes related to their life forms can influence the hydrogen isotope composition of plant leaf water (Shu et al., 2008), besides leaf water enrichment. This represents further potentially uncertainty sources when using leaf wax $\delta^2\text{H}$ to reconstruct leaf water $\delta^2\text{H}$ (and therefore ultimately the $\delta^2\text{H}$ of source water, see below).

The reconstruction of the isotopic composition of leaf water and the day-time relative humidity values using Equations (1)–(3) are also based on the assumption that sugars and *n*-alkanes derive their ^{18}O and ^2H isotope composition from leaf water at the evaporation sites. This is where carbohydrate metabolism and gas exchange take place (e.g. Roden and Ehleringer, 1999; Sachse et al., 2012). Although the leaf water reservoir is isotopically not-uniform (Flanagan et al., 1991; Roden and Ehleringer, 1999; Santrucek et al., 2007), we assume that measured $\delta^{18}\text{O}_{\text{sugars}}$ and $\delta^2\text{H}_{\text{n-alkanes}}$ values are essentially controlled by $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of leaf water at the sites where it undergoes evaporation and that are described by the 'terminal lake' approach applied here. The standard uncertainty of the reconstructed RH_D values [Eq. (3)], derived from the uncertainty propagation law and the analytical uncertainties of the measured $\delta^2\text{H}_{\text{n-alkanes}}$ and $\delta^{18}\text{O}_{\text{sugars}}$, varies between 3 and 20% (Table 1). This uncertainty does not include uncertainties associated with the adopted values of the biosynthetic fractionation factors, and the uncertainties associated with the simplifying assumptions discussed above.

If not measured, the isotopic composition of the source water can be evaluated when the slope of the local leaf water evaporation line (LLEL) is known and the local meteoric water line is defined by direct measurements or can be assumed. The slope of LLEL can be obtained from Eq. (1):

$$S_{\text{leaf-water}} = \frac{\delta_{\text{LW}}^2 - \delta_{\text{SW}}^2}{\delta_{\text{LW}}^{18} - \delta_{\text{SW}}^{18}} = \frac{(1 - h_N)\epsilon_2^* + \Delta\epsilon_2}{(1 - h_N)\epsilon_{18}^* + \Delta\epsilon_{18}} = \frac{\epsilon_2^* + C_k^2}{\epsilon_{18}^* + C_k^{18}} \quad (4)$$

It is worth mentioning here that, with the simplifying assumptions underlying Eqs. (1) and (4), i.e. full isotope steady-state of the leaf water reservoir undergoing evaporation and isotopic equilibrium at ground-level temperature between source water utilized by plants and the local atmospheric water vapor, the slope of LLEL depends only on equilibrium and kinetic fractionation for both isotopes. Slight temperature dependence of the slope is hidden in the temperature dependence of the equilibrium fractionation factors for ^2H and ^{18}O . Assuming present day-time mean surface air temperature for the elevation of Maundi site (+14 °C, cf. Appelhans et al., 2015), the slope of LLEL calculated using Eq. (4) is equal to 2.83. Lowering this temperature by 5 °C, to account for a possible drop of surface air temperature during the glacial period (Sacred Lake, Mt. Kenya: Loomis et al., 2012; Lake Malawi: Woltering et al., 2011; Congo Basin: Weijers et al., 2007; Lake Tanganyika: Tierney et al., 2008; Burundi highlands: Bonnefille et al., 1992), increases the slope derived from Eq. (4) to 2.94. Low LLELs slopes (~2.5 or even lower) were measured in some field studies, too (e.g. Allison et al., 1985; Flanagan et al., 1991). To account for possible uncertainties we therefore generated $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source water}}$ records for

Table 1

Weighted mean $\delta^2\text{H}$ values of leaf wax-derived *n*-alkanes (*n*-C₂₉ and *n*-C₃₁) and weighted mean $\delta^{18}\text{O}$ values of hemicellulose-derived sugars (arabinose, fucose, and xylose). The reported uncertainties represent the weighted mean standard uncertainties. Also calculated/reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$, deuterium-excess_{leaf-water}, mean day-time relative humidities (RH_D), and $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ values are displayed. The reported uncertainties of deuterium-excess_{leaf-water} and RH_D represent expanded uncertainties calculated using the uncertainty propagation law.

Measured				Calculated/Reconstructed						
Depth [cm]	Age [ka BP]	$\delta^2\text{H}_{\text{n-alkanes}}$ [‰]	$\delta^{18}\text{O}_{\text{sugars}}$ [‰]	$\delta^2\text{H}_{\text{leaf-water}}$ [‰]	$\delta^{18}\text{O}_{\text{leaf-water}}$ [‰]	deuterium-excess _{leaf-water} [‰]	RH _D [%]	$\delta^2\text{H}_{\text{source-water}}$ [‰]	$\delta^{18}\text{O}_{\text{source-water}}$ [‰]	
6.5	0.7	-157.3 ± 1.0	36.4 ± 0.9	3.2	9.1	-70.0 ± 7.0	58 ± 7	-41.0	-7.0	
12.5	1.4	-159.3 ± 1.7	34.2 ± 1.0	0.9	7.0	-55.0 ± 8.0	65 ± 8	-35.8	-6.3	
25.0	3.5	-159.3 ± 1.1	34.0 ± 0.9	0.8	6.8	-54.0 ± 7.0	66 ± 7	-35.2	-6.3	
35.0	6.1	-161.0 ± 1.7	32.6 ± 1.2	-1.1	5.5	-45.0 ± 9.0	70 ± 9	-32.4	-5.9	
47.5	7.2	-170.7 ± 0.9	33.6 ± 1.6	-12.7	6.5	-64.0 ± 13.0	60 ± 13	-54.2	-8.6	
60.0	8.1	-165.6 ± 1.0	35.1 ± 1.1	-6.7	7.9	-70.0 ± 9.0	58 ± 9	-51.0	-8.2	
73.5	8.9	-166.6 ± 1.3	36.7 ± 1.2	-7.9	9.4	-83.0 ± 9.0	51 ± 9	-59.2	-9.2	
82.0	9.7	-158.5 ± 1.2	35.7 ± 0.8	1.8	8.5	-66.0 ± 6.0	60 ± 6	-40.5	-6.9	
91.0	10.8	-155.8 ± 0.7	41.3 ± 1.3	5.0	13.9	-106.0 ± 11.0	40 ± 11	-58.3	-9.1	
98.5	11.6	-156.0 ± 1.3	38.2 ± 1.6	4.7	10.9	-82.0 ± 13.0	51 ± 13	-46.2	-7.6	
107.0	12.6	-153.1 ± 1.2	38.3 ± 0.5	8.2	11.0	-80.0 ± 5.0	53 ± 5	-41.3	-7.0	
117.0	13.8	-149.6 ± 0.6	36.9 ± 1.1	12.4	9.6	-64.0 ± 9.0	60 ± 9	-29.1	-5.5	
126.0	14.8	-158.0 ± 1.5	38.0 ± 0.4	2.4	10.7	-84.0 ± 3.0	51 ± 3	-49.1	-8.0	
135.0	15.9	-152.4 ± 1.4	37.5 ± 0.5	9.0	10.2	-73.0 ± 4.0	56 ± 4	-37.0	-6.5	
150.0	17.7	-148.2 ± 0.5	42.0 ± 0.4	14.0	14.6	-103.0 ± 4.0	41 ± 4	-47.7	-7.8	
157.5	18.6	-147.6 ± 1.1	41.0 ± 0.4	14.8	13.6	-94.0 ± 4.0	46 ± 4	-42.1	-7.1	
165.0	19.4	-151.6 ± 0.7	37.8 ± 0.9	10.0	10.5	-74.0 ± 7.0	55 ± 7	-36.7	-6.4	
175.0	20.3	-154.5 ± 1.3	41.3 ± 0.5	6.5	13.9	-105.0 ± 5.0	40 ± 5	-56.1	-8.9	
185.0	21.2	-154.0 ± 0.1	42.0 ± 1.1	7.2	14.6	-110.0 ± 9.0	38 ± 9	-58.1	-9.1	
225.0	29.4	-154.7 ± 0.4	32.9 ± 1.2	6.4	5.8	-40.0 ± 10.0	73 ± 10	-22.3	-4.6	
247.5	33.6	-153.1 ± 1.7	40.9 ± 1.5	8.2	13.5	-100.0 ± 12.0	43 ± 12	-52.0	-8.3	
280.0	38.6	-155.0 ± 1.9	34.1 ± 0.7	5.9	6.9	-49.0 ± 6.0	68 ± 6	-27.7	-5.3	
310.0	43.5	-159.1 ± 1.7	37.7 ± 0.6	1.0	10.4	-83.0 ± 5.0	51 ± 5	-49.9	-8.1	
361.0	52.1	-147.3 ± 1.2	35.2 ± 1.8	15.1	8.0	-49.0 ± 14.0	68 ± 14	-18.3	-4.1	
381.0	55.5	-146.9 ± 2.1	31.9 ± 0.7	15.5	4.8	-23.0 ± 6.0	81 ± 6	-4.2	-2.4	
418.0	61.8	-138.3 ± 1.8	36.5 ± 0.7	25.8	9.2	-48.0 ± 6.0	69 ± 6	-7.1	-2.7	
428.0	63.5	-133.8 ± 1.3	46.7 ± 1.7	31.1	19.2	-122.0 ± 14.0	31 ± 14	-40.7	-6.9	
435.5	64.8	-141.7 ± 0.9	37.2 ± 1.0	21.8	10.0	-58.0 ± 8.0	64 ± 8	-16.3	-3.9	
443.0	66.0	-137.4 ± 1.7	46.8 ± 2.4	26.9	19.3	-127.0 ± 19.0	29 ± 19	-47.5	-7.8	
453.0	67.7	-144.2 ± 1.0	33.3 ± 1.3	18.8	6.1	-30.0 ± 10.0	77 ± 10	-4.9	-2.5	
463.0	69.4	-142.0 ± 1.0	40.4 ± 0.7	21.5	13.1	-83.0 ± 5.0	51 ± 5	-29.8	-5.6	
482.0	72.6	-154.5 ± 1.4	39.1 ± 1.1	6.6	11.8	-88.0 ± 9.0	49 ± 9	-47.0	-7.7	
503.0	76.2	-148.7 ± 0.4	35.0 ± 0.7	13.4	7.8	-49.0 ± 5.0	68 ± 5	-19.9	-4.3	
533.0	81.3	-151.8 ± 0.4	37.9 ± 0.5	9.7	10.6	-75.0 ± 4.0	55 ± 4	-37.4	-6.5	
558.0	85.5	-156.3 ± 0.5	36.2 ± 1.2	4.4	8.9	-67.0 ± 10.0	59 ± 10	-38.4	-6.6	
588.0	90.6	-158.2 ± 0.5	36.2 ± 0.6	2.1	9.0	-70.0 ± 5.0	58 ± 5	-42.1	-7.1	
625.0	96.9	-153.0 ± 0.5	35.6 ± 1.6	8.4	8.4	-59.0 ± 13.0	63 ± 13	-30.1	-5.6	
638.5	99.2	-154.3 ± 0.8	37.6 ± 1.4	6.7	10.4	-76.0 ± 12.0	55 ± 11	-40.9	-7.0	

the Maundi site using three different LLEL slopes: 2.50, 2.75 and 3.00.

The local meteoric water line (LMWL) derived for precipitation collected on the southern slopes of Mt. Kilimanjaro is $\delta^2\text{H} = 8.0 \cdot \delta^{18}\text{O} + 14.8$ (Zech et al., 2015). The intersection of LLEL drawn through the data point representing present-day leaf water at Maundi site (reconstructed using the uppermost sample from 6.5 cm depth; Fig. 2; Table 1), with the LMWL noted above, should provide a good estimation of modern source water at the site. Analogous procedures can be repeated for all reconstructed leaf water data points shown in Fig. 3, leading to a reconstruction of local $\delta^2\text{H}/\delta^{18}\text{O}$ source water values for the entire time span covered by the investigated loess-like paleosol sequence. This in turn can be linked to temporal variations of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of local precipitation at the study area.

It should be noted that the slope and intercept (d-excess) of LMWL for Maundi site most likely have not been constant during the time period covered by the investigated loess-like paleosol sequence. This potentially affects both our reconstructed RH_D values and $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ records for the Maundi site. However, the variability of d-excess of precipitation recorded in Greenland and Antarctic ice cores during the last 100 ka was not larger than $\pm 4\text{‰}$ (Masson-Delmotte et al., 2005; Stenni et al., 2010). This is much smaller compared to the standard uncertainty of the reconstructed d-excess values of leaf water. The latter was calculated using the analytical uncertainties of the measured $\delta^{18}\text{O}_{\text{sugar}}$ and $\delta^2\text{H}_{n\text{-alkane}}$, plugged into the uncertainty propagation law. The calculated uncertainties of the d-excess values of leaf water were in the range from 3.4‰ to 19.5‰ (Table 1).

^2H and ^{18}O isotope composition of the global ocean fluctuated during the Quaternary, responding to climatically-controlled net transfer of water between the global ocean and the cryosphere. During glacial periods, with the global cryosphere at its maximum, the ocean became isotopically enriched. The maximum extent of

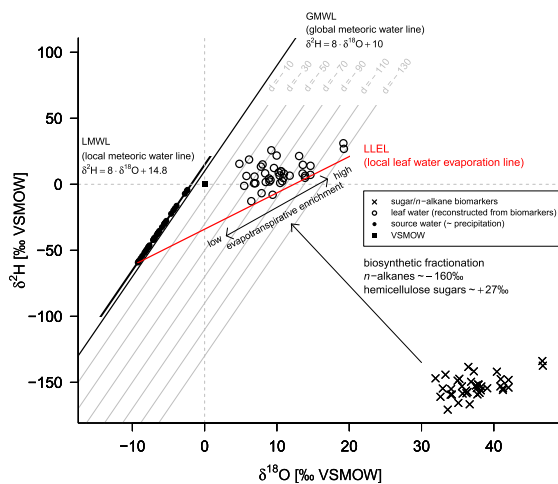


Fig. 3. $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ diagram illustrating the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ approach to reconstruct mean day-time relative humidity values and isotopic composition of plant source water. Data points are plotted for measured $\delta^2\text{H}_{n\text{-alkane}}/\delta^{18}\text{O}_{\text{sugar}}$ values (crosses), for reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf water}}$ values (open circles) and for reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source water/prec}}$ values (filled circles). Accordingly, (i) $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ is calculated from the n -alkane and sugar biomarkers using biosynthetic fractionation factors, (ii) the distance of leaf water to the local meteoric water line (LMWL) – expressed as deuterium-excess – is used to calculate day-time air relative humidity (RH_D), and (iii) $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ is calculated as intersect of the local leaf water evaporation lines (LLEL) with the LMWL (modified according to Zech et al., 2013).

this isotope enrichment was evaluated to be around one per mil for ^{18}O (Schrager et al., 1996, 2002). Since the zero point of the δ scale used for measurements of the isotopic composition of water is defined by the VSMOW standard, which is close to present-day mean isotope composition of the global ocean, appropriate correction is needed when isotopic composition of precipitation (δ_{prec}) for the glacial period is reconstructed and compared with the present-day δ_{prec} values. We applied the correction procedure according to Stenni et al. (2010) which assumes that local factors can be ignored and the d-excess of seawater is set to zero during transitions from glacial to interglacial conditions. The seawater $\delta^{18}\text{O}$ record from Bintanja et al. (2005) was used. This record represents the benthic stack from Lisiecki and Raymo (2005), compiled from 57 globally distributed marine sediment cores, corrected for deep water temperature changes.

4. Results and discussion

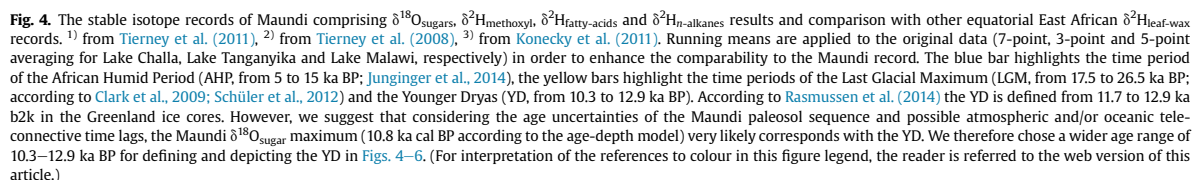
4.1. The Maundi multi-proxy stable isotope records ($\delta^{18}\text{O}_{\text{sugars}}$, $\delta^2\text{H}_{\text{methoxyl}}$, $\delta^2\text{H}_{\text{fatty-acids}}$, $\delta^2\text{H}_{n\text{-alkanes}}$)

The stable isotope records plotted on a time axis are illustrated in Fig. 4. The $\delta^{18}\text{O}_{\text{sugars}}$ values show great variability during the last 100 ka BP ranging from $+31.9$ to $+46.8\text{‰}$ (Fig. 4; Table 1). While the Holocene is characterized by a $\delta^{18}\text{O}_{\text{sugars}}$ minimum ($+32.6$ to $+36.7\text{‰}$), the Younger Dryas (YD) and the Last Glacial Maximum (LGM, from 17.5 to 26.5 ka BP, according to Schuler et al., 2012; Clark et al., 2009; respectively) show distinct $\delta^{18}\text{O}_{\text{sugars}}$ maxima (YD: $+38.2$ – 41.3‰ ; LGM: $+37.8$ – 42.0‰). The pre-LGM portion of the record reveals a pronounced $\delta^{18}\text{O}_{\text{sugars}}$ minimum around 30, 38 and 56 ka BP ($+32.9\text{‰}$, $+34.1\text{‰}$ and $+31.9\text{‰}$) and a pronounced $\delta^{18}\text{O}_{\text{sugars}}$ maximum around 34 ka BP ($+40.9\text{‰}$). The most positive $\delta^{18}\text{O}_{\text{sugars}}$ values occur between 60 and 70 ka BP (peaks with $+46.7\text{‰}$ and $+46.8\text{‰}$).

The $\delta^2\text{H}_{\text{methoxyl}}$ record (Fig. 4), ranging from -160.6 to -74.3‰ (excluding the uppermost data point), has the most positive values between 60 and 73 ka BP (-106.9 to -74.3‰). Although much less pronounced, the YD and the LGM are also characterized by elevated $\delta^2\text{H}_{\text{methoxyl}}$ values (YD: -120.1 to -116.1‰ ; LGM: -115.5 to -108.4‰). $\delta^2\text{H}_{\text{methoxyl}}$ shows only minor variability between 17.5 and 40 ka and a pronounced negative shift for the youngest part of the sequence comprising the modern topsoil (-22.4‰ ; cf. Fig. 4). In contrast to the older sections of the core, the bulk of the topsoil consists mainly of organic matter ($\text{TOC} > 30\%$, whereas most other section show TOC between 5 and 10%; cf. Fig. 2) and potentially includes a large fraction of methoxyl groups e.g. from wood lignin (with more negative $\delta^2\text{H}_{\text{methoxyl}}$ values). Under tropical conditions these components might be readily available for decomposition by wood rooting fungi. However, the reasons for the observed large shift of the $\delta^2\text{H}_{\text{methoxyl}}$ of the topsoil and the higher range shown in the Maundi $\delta^2\text{H}_{\text{methoxyl}}$ values ($\sim 80\text{‰}$; cf. Fig. 4) compared to the $\delta^2\text{H}_{\text{fatty-acid}}$ and $\delta^2\text{H}_{n\text{-alkane}}$ records ($\sim 40\text{‰}$) are currently unclear. In contrast to leaf waxes, which are known to record only a fraction of the leaf water evapotranspirative enrichment (cf. Section 4.3; Gamarra et al., 2016), a higher sensitivity of the methoxyl groups could possibly explain the larger variability.

The $\delta^2\text{H}_{\text{fatty-acid}}$ values range from -161.3 to -126.1‰ (Fig. 4) resembling well the variability of $\delta^2\text{H}_{n\text{-alkane}}$ values ranging from -170.7 to -133.9‰ (Fig. 4; Table 1). There is a good overall agreement between the n -alkanes and the fatty acids ($R = 0.7$; $p < 0.001$). Both records reveal pronounced $\delta^2\text{H}$ minima during the early Holocene, slight maxima during the YD and the LGM, little variability during the pre-LGM period and, like the $\delta^{18}\text{O}_{\text{sugar}}$ and the $\delta^2\text{H}_{\text{methoxyl}}$ records, more positive values between 60 and 70 ka BP.

The uppermost sample of the Maundi loess-like paleosol



respectively. This corresponds to a total difference of $\sim 47\%$ for ~ 2100 m, and thus to a $\delta^2\text{H}_{\text{leaf-wax}}$ lapse rate of $\sim 22.4\text{‰ km}^{-1}$, which is in good agreement with reported $\delta^2\text{H}_{\text{prec}}$ lapse rates ranging from ~ -10 – 40‰ km^{-1} according to [Araguás-Araguás et al. \(2000\)](#). For Mt. Cameroon and Mt. Kilimanjaro, [Gonfiantini et al. \(2001\)](#) and [Zech et al. \(2015\)](#) found $\delta^2\text{H}_{\text{prec}}$ lapse rates of $\sim -14.1\text{‰ km}^{-1}$ (entire altitude span of ca. 4000 m) and $\sim -14.9\text{‰ km}^{-1}$ (above ca. 2000 m a.s.l.), respectively.

Apart from this overall offset, which is well understood, the Maundi $\delta^2\text{H}_{\text{leaf-wax}}$ record has one striking feature in common with Lake Challa and Lake Tanganyika $\delta^2\text{H}_{\text{leaf-wax}}$ records, namely a pronounced $\delta^2\text{H}_{\text{leaf-wax}}$ minimum during the early Holocene as part of the AHP. While this also occurs further north at the Horn of Africa (Tierney and deMenocal, 2013), it is much less pronounced in Lake Malawi and even reversed further south in the catchment of the Zambezi River (Schefuß et al., 2011). This discrepancy dividing central tropical from southern tropical East Africa, has been referred to as the ‘meteorological equator’ or the ‘climate hinge zone’ (e.g. Gasse, 2000; Gasse et al., 2008; Konecny et al., 2011, and references therein), which is represented by a lateral boundary broadly between the Lakes Tanganyika and Malawi. This zone separates the northern equatorial region and the proposed anti-

Compared to other equatorial East African $\delta^2\text{H}_{\text{leaf-wax}}$ records, the Maundi record is characterized by overall more negative $\delta^2\text{H}_{\text{leaf-wax}}$ values (Fig. 4). The mean $\delta^2\text{H}_{\text{fatty-acid}}$ and $\delta^2\text{H}_{\text{n-alkane}}$ values for Maundi are -145‰ and -153‰ , respectively, whereas mean $\delta^2\text{H}_{\text{leaf-wax}}$ values for Lake Challa (Tierney et al., 2011), Lake Tanganyika (Tierney et al., 2008) and Lake Malawi (Konecky et al., 2011) are -105‰ , -104‰ and -108‰ , respectively. This can be attributed to differences in elevation of those sites: Maundi is located at 2780 m a.s.l., whereas Lake Challa, Lake Tanganyika and Lake Malawi are located at 880 m a.s.l., 773 m a.s.l. and 474 m a.s.l.,

phase changes in the south, both insolation-driven paleoprecipitation regimes (e.g. Patridge et al., 1997; Barker et al., 2002). Higher amplitude in the Lake Tanganyika $\delta^2\text{H}_{\text{leaf-wax}}$ variability since ~10 ka BP compared to those of Lake Challa and Maundi might indicate a feedback mechanism associated with a variable Lake Kivu (1460 m a.s.l.) discharge (Cohen et al., 1997; Felton et al., 2007). Accordingly, higher precipitation amounts might result in more terrestrial organic material formed at higher elevations being transported from the Lake Kivu catchment via the Ruzizi River into Lake Tanganyika, thus leading to more negative $\delta^2\text{H}_{\text{leaf-wax}}$ values in the sediments of this lake. However, it is challenging to derive more detailed paleoclimate implications from the Maundi $\delta^2\text{H}_{\text{leaf-wax}}$ records alone due to the large number of processes influencing them: e.g. moisture transport history (Konecny et al., 2011; Tierney et al., 2011), precipitation amount (Schefuß et al., 2011, 2005; Tierney et al., 2008; Tierney and deMenocal, 2013) and leaf water enrichment caused by evapotranspiration (Kahmen et al., 2013; Tierney et al., 2010). Therefore, the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{--}\delta^{18}\text{O}_{\text{sugar}}$ approach seems to be a valuable tool for (i) quantifying this leaf water enrichment effect and (ii) getting the relative day-time humidity and the isotopic composition of plant source water as new paleoclimate proxies.

4.3. Day-time relative humidity history at Maundi

The reconstructed d-excess values of leaf water for the Maundi profile range from -127 to -23‰ (Table 1), with the lowest value recorded at ca. 66 ka and the highest at ca. 55 ka. Changes of RH_D

mirror the changes of the d-excess_{leaf-water}. The biomarker-based day-time relative humidity record [RH_D ; derived from Eq. (3)] from the Maundi paleosol sequence reveals large variability for the last ~100 ka BP. RH_D ranges from 29% to 81% (Fig. 5). The modern topsoil sample (6.5 cm depth) yields an RH_D value of 58% ($\pm 3.7\%$; Table 1). For comparison, Pepin et al. (2010) reported for this elevation on the southern slopes of Mt. Kilimanjaro, a higher mean annual free-air relative humidity of approximately 65%. The measured ground-level mean annual relative humidity values are reported to be much higher, between 88% and 96% (Duane et al., 2008; Appelhans et al., 2015; cf. Fig. 1c). This apparent discrepancy is addressed below.

The proposed coupled ' $\delta^2\text{H}_{n\text{-alkane}}\text{--}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer' is prone to uncertainties resulting from analytical uncertainties of $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ measurements (Fig. 4) and the uncertainties of $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ biosynthetic fractionation factors. Although we consider the temperature dependence of the biosynthetic fractionation factors to be negligible (Zech et al., 2014a), it is very likely that an ϵ_{bio} value of $+27\text{‰}$ for reconstructing $\delta^{18}\text{O}_{\text{leaf-water}}$ from arabinose and xylose underestimates the true ϵ_{bio} value (cf. Zech et al., 2014b, and discussion therein). A slightly higher ϵ_{bio} value of $+29\text{‰}$ would increase the reconstructed RH_D for the uppermost sample of Maundi from 58% to 66%. Similarly, an increase of ϵ_{bio} for deuterium by 10‰ (from -160‰ to -170‰) would lead to a corresponding increase of RH_D by 6%. Therefore, the reconstructed RH_D values should be considered as minimum estimates based on these considerations.

On the other hand, it might be worth trying to account for

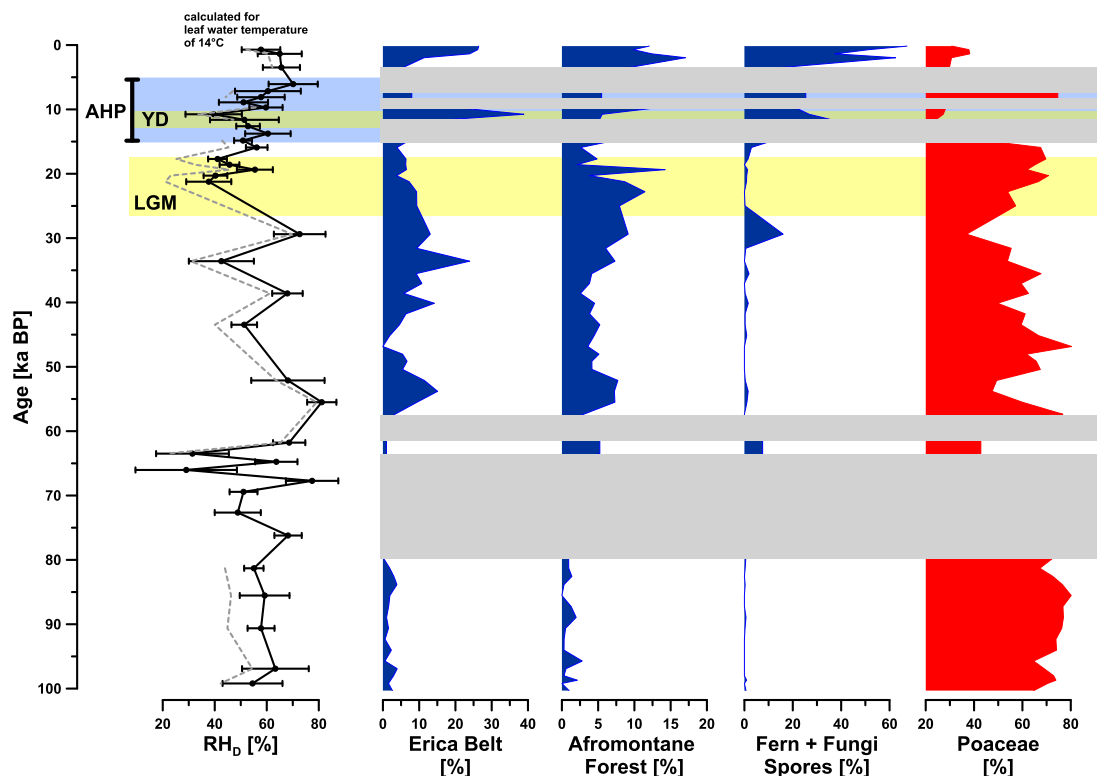


Fig. 5. Reconstructed day-time relative humidity record (RH_D) for the Maundi paleosol sequence. Error bars indicate expanded uncertainties derived from the uncertainty propagation law. Also pollen records for Maundi site are shown (Schüler et al., 2012).

variable contributions of grass-derived biomarkers, because it is reported that grass-derived *n*-alkane and sugar biomarkers are not (fully) sensitive for recording the evapotranspirative enrichment signal of leaf water (Helliker and Ehleringer, 2002; McInerney et al., 2011). This effect can be presumably explained with the grass leaf growth from a basal intercalary meristem, where leaf water enrichment is diluted by non-enriched stem water. Based on findings of Helliker and Ehleringer (2002) we assumed that 35% of the leaf water enrichment is lost during hemicellulose sugar biosynthesis; and based on findings of Gamarra et al. (2016) and Kahmen et al. (2013) we assumed that 50% of the leaf water enrichment is lost/not seen during *n*-alkane biosynthesis of leaves. Assuming furthermore that the *Poaceae* pollen concentration (Fig. 5) corresponds to the *Poaceae* biomarker contribution (which is surely not correct but may serve as rough approximation and is without alternative at the current state of knowledge), an isotope mass balance calculation can be applied. The thus “corrected” RH_D record (albeit underlying many assumptions and uncertainties) yields on average 6.9% lower RH_D values (Fig. 5, grey dotted line, maximum offset 17.2% around 85 ka BP) than the uncorrected RH_D record. Nevertheless, these consideration and results overall corroborate the robustness of our paleohygrometer approach and even amplify most of the uncorrected RH_D variability (Fig. 5).

Furthermore, as discussed above, the proposed paleohygrometer is a proxy for mean day-time values of air relative humidity, i.e. when stomata are open, the transpiration process is at its maximum and biomarker synthesis takes place (e.g. Tuthorn et al., 2015). The relative humidity of air on the slopes of Mt Kilimanjaro exhibits strong altitudinal gradients with the maximum coinciding well with the altitudinal precipitation maximum (Fig. 1c). Also, it is subject to pronounced seasonal as well as diurnal variability (Appelhans et al., 2015; Duane et al., 2008; Pepin et al., 2010). While the seasonal variability of relative humidity is controlled by the migration of the ITCZ, the strong diurnal variability of this parameter at Maundi site is caused by specific circulation patterns of the local atmosphere, with upslope moisture transport during the day and downslope transport and drying at night (see Fig. 11 in Pepin et al., 2010). We suggest that the Maundi RH_D record depicted in Fig. 5 reflects the long-term variability of mean day-time values of air relative humidity.

Finally, the apparent offset between the reconstructed RH_D values and the ground-level instrumental data, seen at Maundi site, may largely stem from the fact that when biomarkers are biosynthesized predominantly within the canopy (Zech et al., 2015) they will record canopy-level rather than ground-level relative humidity values. As demonstrated by Graham et al. (2014) the differences in morning-time (9 a.m.–12 p.m.) relative humidity recorded in tropical forest at ground-level (0–5 m), and at canopy-level (>5 m), may easily reach 20%. This would imply grasses to record the higher ground-level relative humidity as opposed to trees, which incorporate the lower canopy-level relative humidity values. This effect points in the same direction as the correction made above (due to relatively insensitive grasses concerning recording leaf water evapotranspirative enrichment). Considering high uncertainties associated to this correction, a further adjustment seems not to be needed or is already covered.

The most outstanding feature of the lower part of the Maundi RH_D record shown in Fig. 5 is the reduction of this parameter during the period from ca. 70 to 60 ka BP. This extreme drought period is corroborated by the absence of pollen, which is interpreted in terms of poor pollen preservation due to dry conditions (Schüler et al., 2012). Within dating uncertainties, this pronounced drought period might correspond to low stands of Lake Malawi (Scholz et al., 2007) and Lake Challa (Moernaut et al., 2010).

Although the resolution of Maundi RH_D record is relatively low

from 60 to 25 ka BP, the recorded variability of this parameter is also corroborated by the Maundi pollen results (Fig. 5). The two pronounced RH_D maxima around ~55 and during the pre-LGM around ~30 ka BP coincide with *Poaceae* minima, whereas humid-indicating taxa of the *Erica* belt, the Afromontane forest and fern and fungi spores reveal maxima. By contrast, the onset of the LGM is characterized by a marked decline of the latter taxa and a marked increase of the *Poaceae* taxa, in line with RH_D minima; a first one from ~22 to 20 ka BP and a second one around ~17.5 ka BP (Fig. 5). The first minimum coincides with a lake level drop of Lake Tanganyika of 300 m at around 21 ka BP (Gasse et al., 1989) or at least ~260 m during the LGM (32–14 ka BP; McGlue et al., 2007), while Lake Victoria was nearly desiccated at that time (Talbot and Livingstone, 1989). Within dating uncertainties of the investigated loess-like paleosol sequence, the second arid spell is consistent with one of the most extreme lake-level low stands reported for Lake Challa, dated to ~17.0–16.4/16.9–16.3 ka BP (Moernaut et al., 2010; Verschuren et al., 2009).

A late glacial shift to more humid conditions is evident both in the reconstructed Maundi RH_D record and the pollen spectra. This shift is in agreement with the onset of the AHP and equatorial East African lake transgressions (Gasse, 2000; Junginger et al., 2014; and references therein) as well as rainfall modeling results (Otto-Bliesner et al., 2014). A pronounced arid spell during the YD is only visible in the RH_D record, not in pollen, which partly suffers from pollen preservation in the upper part of the investigated loess-like paleosol sequence. Such pronounced arid climatic conditions during the YD seem to have been a widespread phenomenon in East Africa (Gasse et al., 2008) and were for instance reported for the close-by sedimentary record of Lake Challa (Tierney et al., 2011; Verschuren et al., 2009).

The reconstructed RH_D values increase during the Holocene and reach a maximum during the middle Holocene. This is in accord with pollen results showing a *Poaceae* maximum and still moderate abundance of *Erica* and Afromontane forest taxa during the early Holocene. Only during the middle and the late Holocene, when the AHP ended, the RH_D record stays still high and the Afromontane forest, fern and fungi taxa reach their maximum indicating very humid conditions. The same Holocene climate history is recorded in the WeruWeru pollen study site, located in the montane forest at an elevation of 2650 m on the southern slopes of Mt. Kilimanjaro. According to Schüler (2013), drought tolerant *Cassipourea* forests prevailed here during the early Holocene. Over the course of the Holocene montane forest taxa typical for the wetter southern slopes, which also form the forests today, became more abundant (Schüler, 2013). Lake levels in equatorial East African, reached their maximum during the early Holocene and dropped over the course of the Holocene (Gasse, 2000; Junginger et al., 2014; and references therein). This apparent discrepancy is addressed in Section 4.5.

The uppermost sample, representing the modern topsoil, suggests again more arid climatic condition (Fig. 5). This is well in agreement with climate and environmental observations indicating considerably decreasing amounts of precipitation and relative humidity on Mt. Kilimanjaro during the last century (Hemp, 2005; Mölg et al., 2009).

4.4. Reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ and its paleoclimate implications

Apart from reconstructing mean day-time relative humidity, the coupled $\delta^2\text{H}_{\text{n-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ approach allows also the assessment of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of water used up by plants being the source of biomarkers analysed in this study (cf. Fig. 3). In order to address potential uncertainty of the reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$

values associated with the slope of the local leaf water evaporation line (LLEL), we used three different values of this slope: 2.50, 2.75 and 3.00. The values of $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ reported in Table 1 were derived with the slope of LLEL equal to 2.75. The reconstructed Maundi $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ records are shown in Fig. 6. For comparison, the figure depicts also the RH_D record and the $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ record.

The modern topsoil sample of Maundi yields $\delta^2\text{H}_{\text{source-water}}$ and $\delta^{18}\text{O}_{\text{source-water}}$ values of -41‰ and -7‰ , respectively using a LLEL slope of 2.75 (Fig. 6; Table 1). A direct comparison with present-day isotope precipitation signal at this site cannot be made due to the lack of data. Modern $\delta^2\text{H}/\delta^{18}\text{O}_{\text{prec}}$ values recorded on south-western slopes of Mt. Kilimanjaro at the elevation of 2800 m a.s.l., approximately 30 km to the west of Maundi, are significantly less negative: $\sim -22\text{‰}$ and $\sim -5\text{‰}$, respectively (Zech et al., 2015). This apparent offset may stem from local differences in altitude gradient of $\delta^2\text{H}/\delta^{18}\text{O}_{\text{prec}}$, induced by differences in atmospheric circulation and/or local moisture recycling (cf. discussion in Section 4.3). It is worth noting that the vertical extent of the montane rainforest belt on the south-eastern slopes of Mt. Kilimanjaro is

significantly smaller than that observed at southern and south-western slopes (Hemp and Beck, 2001), which may result in a lower degree of moisture recycling and a higher $\delta^2\text{H}/\delta^{18}\text{O}_{\text{prec}}$ altitude gradient at the Maundi location.

It is also apparent from Fig. 6 that the reconstructed Maundi $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ records do not closely resemble the corresponding $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ records (and thus the biomarker records). The entire $\delta^{18}\text{O}_{\text{source-water}}$ record has a weak negative correlation with $\delta^{18}\text{O}_{\text{sugars}}$ ($R = -0.26$; $p = 0.001$); the correlation is positive for $\delta^2\text{H}_{\text{source-water}}$ and $\delta^2\text{H}_{n\text{-alkanes}}$, yet the coefficient of correlation is also quite low ($R = 0.26$; $p < 0.001$). This suggests that changes in $\delta^{18}\text{O}_{\text{sugar}}$ and $\delta^2\text{H}_{\text{leaf-wax}}$ records are to some extent decoupled from changes in $\delta^2\text{H}/\delta^{18}\text{O}_{\text{prec}}$ at least in those cases where paleohumidity, and thus the isotopic enrichment of leaf water, is highly variable.

4.5. Controls on paleohumidity and $\delta^2\text{H}/\delta^{18}\text{O}$ of paleoprecipitation on the southeastern slopes of Mt. Kilimanjaro

The Maundi paleohumidity record shows some broad

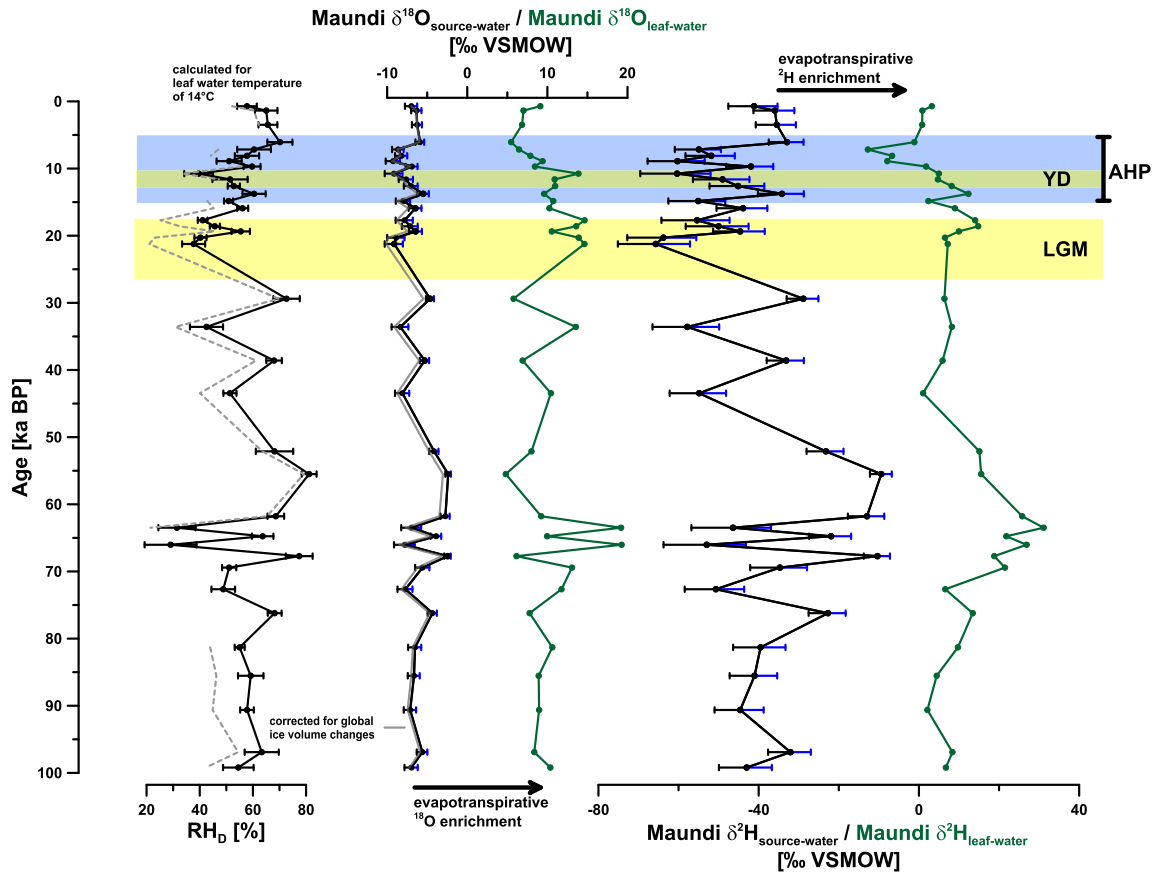


Fig. 6. Climatic records reconstructed for Maundi site. Left: Mean day-time relative air humidity (RH_D). Error bars indicate expanded uncertainties derived from the uncertainty propagation law. The grey dotted line depicts a “corrected” RH_D record, that accounts for vegetation changes (contribution of grass-derived n -alkane and sugar biomarkers, see text Section 4.3 for details). Middle: Record of $\delta^{18}\text{O}_{\text{source-water}}$ reconstructed using local leaf water evaporation line slope (LLEL) of 2.75 (blue and black error bars represent the confidence interval calculated using LLEL slopes of 2.50 and 3.00, respectively) and $\delta^{18}\text{O}$ values of leaf water. The $\delta^{18}\text{O}_{\text{source-water}}$ curve corrected for ice-volume effect is also shown (in grey). $\delta^{18}\text{O}_{\text{leaf-water}}$ is shown in green. Right: Record of $\delta^2\text{H}_{\text{source-water}}$ reconstructed for a LLEL slope of 2.75 (blue and black error bars represent LLEL slopes of 2.50 and 3.00, respectively), corrected for ice-volume effect. $\delta^2\text{H}_{\text{leaf-water}}$ is shown in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

similarities to previously published regional paleoprecipitation records (East African lake-level history and local pollen records; cf. Section 4.3). This suggests that one important factor for Maundi RH_D record is the overall regional moisture availability that is associated with the precipitation provided by the East African monsoon system. However, as mentioned above, discrepancies appear between Maundi RH_D record and the lake stand record. Lake level high-stands during the early Holocene are followed by lake level regressions during the late Holocene, whereas Maundi RH_D values reach their maximum only in the middle Holocene, in line with the pollen records from the same site (Schüler et al., 2012) and the WeruWeru study site at 2650 m a.s.l. (Schüler, 2013). This apparent discrepancy can be reconciled by taking into account local factors. Mölg et al. (2009) speculated that the altitudinal belt of maximum precipitation, which is located at present at about ~2200 m a.s.l. on the southern slopes of Mt. Kilimanjaro, might have migrated vertically in the past. We propose that this belt, and thus also the belt of maximum RH_D, migrated uphill along the slopes of Mt. Kilimanjaro in direction to the Maundi study site at ~2780 m a.s.l. over the course of the Holocene. The vertical migration of the belt could be controlled by increasing moisture availability and also by variable inversion – a concept described by Augstein et al. (1974) as ‘trade wind inversion’ for the Atlantic Ocean. During the early Holocene, the trade wind inversion of the southeast trades was presumably strongly developed due to the 30°N summer insolation maximum and thus enhanced latent heat transfer into the higher atmospheric layers in the northern African tropics. As a consequence of the well-developed trade wind inversion during the early Holocene, the diurnal thermal circulation on Mt. Kilimanjaro, and thus also the cloud formation in the subalpine zone where the Maundi study site is located, was suppressed (cf. Fig. 11 in Pepin et al., 2010). However, the shifts in the Maundi RH_D record does not follow the maxima in equatorial or the Northern Hemisphere summer insolation. The prominent drought period (ca. 70–60 ka BP, Fig. 5) cannot be explained via orbital forcing either and its cause remains unclear.

Concerning the factors controlling variability of $\delta^2\text{H}/\delta^{18}\text{O}$ of paleoprecipitation, a straightforward application of the ‘amount effect’, as we know it from modern precipitation, may not be applicable for the Maundi precipitation record. Apart from the fact that a long-term/interannual isotope ‘amount effect’ in tropical precipitation is by no means clear from the instrumental data (Rozanski et al., 1996), an apparent positive correlation of RH_D record with $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ is visible in the presented data (Fig. 6, $R = 0.60$; $p < 0.001$). This effect could be generated by the local vegetation, particularly the montane rainforest. The isotopic composition of transpired moisture is similar to that of plant source water under steady-state conditions (e.g. Bariac et al., 1991; Flanagan et al., 1991), thus being isotopically much heavier than the vapor of marine origin. Along the southern slopes of Mt. Kilimanjaro, $\delta^2\text{H}_{\text{prec}}$ has a local maximum in the montane rainforest at about 2000–2200 m a.s.l. (Zech et al., 2015). This maximum can be interpreted in terms of an increasing proportion of transpired, i.e. isotopically enriched moisture. At Mt. Kilimanjaro, the montane rainforest is an important atmospheric moisture source under present climatic conditions (Pepin et al., 2010), and we suggest it contributed also in the past to the precipitation at Maundi site, with the importance varying in accordance with the fluctuation of its size and vertical extent. This could explain that maxima of the reconstructed RH_D record generally coincide with an increase in Afromontane pollen taxa and more isotopically enriched source water (precipitation). During arid periods the Afromontane forest belt descended and/or diminished, which resulted in more isotopically depleted precipitation at the Maundi site. A rough

assessment based on modern $\delta^2\text{H}_{\text{prec}}$ transect presented in Fig. 3b of Zech et al. (2015) shows that this local effect could be in the order of 20‰ and 2.5‰ for $\delta^2\text{H}_{\text{prec}}$ and $\delta^{18}\text{O}_{\text{prec}}$, respectively.

Apart from the local effect associated with montane rainforest, changes in the isotopic composition of precipitation at Maundi site could be also influenced by regional effects associated with the regional biosphere as an important factor controlling moisture recycling over the East African continent. Dry conditions at the vicinity of Maundi site, as well as further south and southeast of Mt. Kilimanjaro, towards the coast of the Indian Ocean, most probably resulted in scarce vegetation and consequently a reduced source of isotopically heavy water vapor for the regional atmosphere. This would mean that even reduced precipitation during dry periods might lead to higher effective rainout of moist air masses of marine origin and the resulting depletion of heavy isotope content in precipitation falling on the southern and eastern slopes of Mt. Kilimanjaro. The importance of the biosphere-modulated recycling of water for continental water balance is clearly seen in present-day Europe. The gradient of $\delta^2\text{H}/\delta^{18}\text{O}_{\text{prec}}$ across the European continent during summer is nowadays significantly weaker than it would be without the transpiration flux operated on continental scale (Rozanski et al., 1982). Similar modulation of the extent of continental effect in $\delta^2\text{H}/\delta^{18}\text{O}_{\text{prec}}$ in response to varying biospheric feedback, can be expected along the passage of East African monsoon all the way to the Maundi site.

5. Conclusions

The Maundi loess-like paleosol sequence provides a valuable paleoclimate/environmental archive roughly comprising the last 100 ka. We summarize our results as follows:

- The records of $\delta^{18}\text{O}_{\text{sugars}}$, $\delta^2\text{H}_{\text{methoxy}}$, $\delta^2\text{H}_{\text{fatty-acids}}$ and $\delta^2\text{H}_{\text{n-alkanes}}$ reveal similar patterns. The periods from ~70 to 60 ka, the LGM and YD are characterized by relatively positive δ values, whereas during the Holocene relatively negative δ values occurred. The important differences comprise higher variability of the $\delta^{18}\text{O}_{\text{sugar}}$ record, timing of the Holocene minima, and larger fluctuations in $\delta^2\text{H}_{\text{methoxy}}$ values, as well as a shift to very negative values in the modern topsoil.
- Comparison of the Maundi $\delta^2\text{H}_{\text{leaf-wax}}$ record with other available equatorial East African $\delta^2\text{H}_{\text{leaf-wax}}$ records (Lake Challa, Lake Tanganyika) shows that they all reveal distinct $\delta^2\text{H}_{\text{leaf-wax}}$ minima within the AHP, but the pattern, timing and amplitude are somewhat different. The most striking difference among them is a clear regional ‘altitude effect’ resulting in the Maundi $\delta^2\text{H}_{\text{leaf-wax}}$ record being shifted to more negative δ values with respect to those recorded in lake sediments.
- The various influences on $\delta^2\text{H}_{\text{leaf-wax}}$ records from East Africa (e.g. moisture transport history, precipitation amount, evapo-transpirative enrichment) challenge a straightforward interpretation in terms of paleoclimate. We suggest that the coupled $\delta^2\text{H}_{\text{n-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ approach could provide more robust proxies, namely the day-time relative humidity (RH_D) and the isotopic composition of plant source water ($\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$), by accounting for the leaf water enrichment.
- The reconstructed day-time relative humidity record for Maundi site is generally in agreement with the Maundi pollen results (Schüler et al., 2012), suggesting arid climatic conditions from ~70 to 60 ka, during the LGM and the YD, whereas the pre-LGM, the Late Glacial and the middle and late Holocene were characterized by more humid climatic conditions. Apart from the overall regional moisture availability, we suggest that the intensification/weakening of the trade wind inversion, which

affects the diurnal montane atmospheric circulation on the slopes of Mt. Kilimanjaro, is a local process, which may influence changes of relative humidity recorded at the Maundi study site.

- The results of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{--}\delta^{18}\text{O}_{\text{sugar}}$ approach presented here caution against directly interpreting $\delta^2\text{H}_{\text{leaf-wax}}$ (as well as $\delta^{18}\text{O}_{\text{sugar}}$) records as proxies of $\delta^2\text{H}_{\text{prec}}$ ($\delta^{18}\text{O}_{\text{prec}}$). Changes in relative humidity and the resulting variations in isotopic evapotranspirative enrichment of leaf water (Fig. 6) can mask changes of $\delta^2\text{H}_{\text{prec}}$ ($\delta^{18}\text{O}_{\text{prec}}$).
- Strong positive correlation between RH_D and $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$, observed at the Maundi site on millennial time scale (Fig. 6), suggests that a straightforward application of the ‘amount effect’, as we know it from modern precipitation, cannot explain the reconstructed isotopic composition of local precipitation. Effects associated with the impact of local and regional biosphere on the isotopic composition of atmospheric moisture and precipitation need to be called on to understand $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ records on millennial time scale. They involve expansion/shrinking or complete disappearance of montane rainforest on the southeastern slopes of Mt. Kilimanjaro as well as expansion/shrinking of regional vegetation cover in response to climatic changes in the region.

Clearly, further work is needed to improve our understanding of isotope biomarkers records preserved in continental archive. This is particularly true for the East African region.

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A sugar biomarker proxy for assessing terrestrial versus aquatic sedimentary input



Johannes Hepp^{a,b,*}, Max Rabus^c, Tobias Anhäuser^d, Tobias Bromm^b, Christian Laforsch^c, Frank Sirocko^e, Bruno Glaser^b, Michael Zech^{b,f}

^a Chair of Geomorphology and BayCEER, University of Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth, Germany

^b Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin-Luther University Halle-Wittenberg, von-Seckendorff-Platz 3, D-06120 Halle, Germany

^c Animal Ecology I and BayCEER, University of Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth, Germany

^d Institute of Earth Sciences, Ruprecht Karls University Heidelberg, Im Neuenheimer Feld 234-236, D-69120 Heidelberg, Germany

^e Institute of Geosciences, Johannes Gutenberg University Mainz, J.-J.-Becher-Weg 21, D-55128 Mainz, Germany

^f Institute of Geography, Chair of Landscape- and Geoecology, Technical University of Dresden, Helmholtzstrasse 10, D-01062 Dresden, Germany

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ABSTRACT

One of the most important and at the same time most challenging issues in paleolimnological research is the differentiation between terrestrial and aquatic sedimentary organic matter (OM). We therefore investigated the relative abundance of the sugars fucose (fuc), arabinose (ara) and xylose (xyl) from various terrestrial and aquatic plants, as well as from algal samples. Algae were characterized by a higher abundance of fucose than vascular plants. Our results and a compilation of data from the literature suggest that fuc/(ara + xyl) and (fuc + xyl)/ara ratios may serve as complementary proxies in paleolimnological studies for distinguishing between terrestrial and aquatic sedimentary OM.

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1. Introduction

Lake sediments are valuable, often continuous and potentially high resolution, archives for studying past environmental and climate changes. This is highlighted by methods developed during the last few decades based on compound specific ¹⁸O and ²H results from hemicellulose/polysaccharide derived sugars and from leaf wax/aquatic plant derived *n*-alkanes, respectively (e.g. Rach et al., 2014; Zech et al., 2014b). Thereby, often one of the most crucial questions and challenges (Meyers and Ishiwatari, 1993) is to identify whether the origin of the sedimentary organic matter (OM) is allochthonous (terrestrial) or autochthonous (aquatic). The issue is typically addressed by way of different approaches and proxies in paleolimnological studies.

For instance, the C/N ratio of sedimentary total OM is frequently used to distinguish between algal and land derived material. This

proxy is based on the notion that land plants generally show markedly higher C/N values than lacustrine plants (Meyers and Ishiwatari, 1993). Further differentiation between input of C₃ vs. C₄ land plants is possible on the basis of the stable carbon isotopic composition (δ¹³C; Meyers, 1994; Meyers and Lallier-Vergès, 1999). However, both δ¹³C and C/N values of terrestrial OM are affected by mineralization and degradation, resulting in more positive δ¹³C values and lower C/N ratio (e.g. Zech et al., 2007). The latter could lead to a misinterpretation of soil OM transported by soil erosion into lacustrine systems as being aquatic-derived sedimentary OM. Similarly, other studies have demonstrated that the hydrogen index and oxygen index (HI, OI; derived from Rock-Eval analysis) may provide valuable information about the origin of sedimentary OM (Talbot and Livingstone, 1989; Meyers and Lallier-Vergès, 1999; Mügler et al., 2010). However, the indices are strongly affected by oxidation of the sedimentary OM and in addition strongly depend on the quality of terrestrial OM (waxy OM vs. cellulose rich OM; Lüniger and Schwark, 2002). Additional information and clarification about terrestrial vs. aquatic OM origin may be provided by lipid biomarkers. This is realized mainly by

* Corresponding author at: Chair of Geomorphology and BayCEER, University of Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth, Germany. Tel.: +49 921 55 2172.

E-mail address: johannes-hepp@gmx.de (J. Hepp).

Table 1

Samples and concentrations of fucose (fuc), arabinose (ara) and xylose (xyl), sum of sugars (ara + fuc + xyl) and fuc/(ara + xyl) ratio. Median values of each group are given in the respective group row.

Sample	Name	Study site/origin	Fucose (mg/g sample)	Arabinose (mg/g sample)	Xylose (mg/g sample)	Sum of sugars (ara + fuc + xyl) (mg/g sample)	Fuc/(ara + xyl)
Terrestrial plants							
BS_Acer	Maple	Lake Bichlersee	0.54	26.20	21.75	50.42	0.01
BS_Buche	Beech	Lake Bichlersee	1.58	31.85	43.73	77.17	0.02
BS_Fichte	Spurce	Lake Bichlersee	0.41	32.93	66.98	99.91	0.00
BS_Gras1	Grass unspec.	Lake Bichlersee	0.00	30.77	21.75	52.93	0.01
BS_Gras2	Grass unspec.	Lake Bichlersee	0.00	268.45	512.35	780.81	0.00
BS_Tanne	Pine	Lake Bichlersee	0.00	31.80	105.99	137.79	0.00
GM6	Leaves sample (under water)	Lake Gemündener Maar	0.59	34.59	19.71	54.90	0.01
GM8	Leaves sample (shore area)	Lake Gemündener Maar	1.58	55.40	62.01	119.00	0.01
B1	Blackberry	Lake Holzmaar	0.47	22.13	27.82	50.42	0.01
B2	Blackberry	Lake Holzmaar	0.54	10.49	10.68	21.71	0.02
B3	Blackberry	Lake Holzmaar	0.38	10.28	12.77	23.42	0.01
B7	Blackberry	Lake Holzmaar	1.02	20.43	26.05	47.50	0.02
B8	Blackberry	Lake Holzmaar	0.17	9.57	19.20	28.95	0.00
HG1	Rose hip	Lake Holzmaar	0.64	12.07	14.47	27.18	0.02
HG2	Rose hip	Lake Holzmaar	0.90	15.23	7.90	24.02	0.03
			0.87	26.20	19.99	47.06	0.01
Emergent plants							
BS1	Reed	Lake Bichlersee	0.00	54.33	98.35	144.73	0.00
BS4	Juncus	Lake Bichlersee	0.00	179.24	608.63	787.87	0.00
BS5	Carex roots	Lake Bichlersee	0.00	39.16	88.34	127.51	0.00
BS6	Carex leaf	Lake Bichlersee	0.00	55.07	72.45	127.52	0.00
BS9	Emergent plant unspec.	Lake Bichlersee	0.00	22.10	31.94	54.04	0.00
GM2	Cane green	Lake Bichlersee	1.02	22.77	12.12	35.91	0.03
GM3	Cane dead	Lake Gemündener Maar	0.00	232.45	452.81	685.25	0.00
GM4	Iris pseudacorus green	Lake Gemündener Maar	0.00	192.54	445.21	637.75	0.00
GM5	Iris pseudacorus death	Lake Gemündener Maar	0.00	17.35	37.50	54.84	0.00
HM_S1	Reed	Lake Holzmaar	0.00	57.25	136.54	193.79	0.00
HM_S2	Reed	Lake Holzmaar	0.00	20.81	38.56	59.37	0.00
HM_S3	Reed	Lake Holzmaar	0.00	23.70	57.70	81.40	0.00
P3	Reed	Pond near Rosenheim	0.00	53.58	108.36	161.94	0.00
P5	Carex	Pond near Rosenheim	0.00	108.91	390.74	499.66	0.00
			0.00	98.59	195.72	294.31	0.00
Mosses							
BS7	Moss unspec.	Lake Bichlersee	0.54	17.66	18.45	36.65	0.02
P2	Moss unspec.	Pond near Rosenheim	0.28	18.45	20.48	39.21	0.01
			0.81	16.86	16.41	34.08	0.02
Submerged aquatic plants							
BS10	Water lily	Lake Bichlersee	0.65	12.10	12.47	25.31	0.02
BS11	Submerge plant unspec.	Lake Bichlersee	0.46	15.79	43.00	59.25	0.01
GM1	Submerge plant unspec.	Lake Gemündener Maar	1.66	11.11	10.17	22.95	0.08
GM7	Water lily	Lake Gemündener Maar	0.42	27.09	33.92	61.43	0.01
HF1	Submerge plant unspec.	Lake Hofstätter See	0.46	11.93	62.04	74.43	0.01
AL1	Stoneworts	Lake Holzmaar	0.11	24.92	29.62	54.66	0.00
AL2	Stoneworts	Lake Holzmaar	0.84	12.27	6.55	19.65	0.03
AL3	Stoneworts	Lake Holzmaar	1.73	14.33	11.62	27.68	0.05
PP2	Submerge root felt	Lake Panch Pokhari	1.08	9.09	4.48	14.65	0.06
P4	Elodea unspec.	Pond near Rosenheim	0.89	8.54	13.32	22.75	0.04
			0.18	9.79	7.87	17.85	0.01
Algae							
BS2	Algae	Lake Bichlersee	7.50	16.01	16.20	34.50	0.34
BS3	Algae	Lake Bichlersee	7.50	17.91	4.15	29.56	0.34
BS8	Algae	Lake Bichlersee	11.74	12.86	9.90	34.50	0.52
PP1	Algae crust	Lake Panch Pokhari	1.80	16.01	16.20	34.01	0.06
P1	Algae	Pond near Rosenheim	3.24	12.09	20.59	35.91	0.10
			50.77	32.02	52.73	135.53	0.60

(continued on next page)

Table 1 (continued)

Sample	Name	Study site/origin	Fucose (mg/g sample)	Arabinose (mg/g sample)	Xylose (mg/g sample)	Sum of sugars (ara + fuc + xyl) (mg/g sample)	Fuc/(ara + xyl)
Green algae							
Bot	<i>Botryococcus braunii</i>	Cultivated	2.36	1.55	2.20	5.50	0.33
NanoJim16	<i>Nannochloropsis limnetica</i>	Cultivated	4.56	6.45	8.18	19.19	0.24
NanoJim2	<i>Nannochloropsis limnetica</i>	Cultivated	2.14	1.18	1.35	4.67	0.65
NanoJim9	<i>Nannochloropsis limnetica</i>	Cultivated	3.38	4.79	3.09	11.25	0.33
Ped	<i>Pediastrum boryanum</i>	Cultivated	4.06	2.83	2.20	9.08	0.62
Ped.bo	<i>Pediastrum boryanum</i>	Cultivated	0.00	1.41	0.86	2.26	0.00
Acut (former Scene)	<i>Acutodesmus obliquus</i>	Cultivated	0.57	1.55	2.00	4.12	0.12
Diatoms							
Fra	<i>Fragilaria crotonensis</i>	Cultivated	2.36	0.37	2.77	5.50	0.60
Gom	<i>Gomphonema parvulum</i>	Cultivated	3.28	1.00	5.68	10.14	0.41
Gom6315	<i>Gomphonema parvulum</i>	Cultivated	15.73	0.87	2.93	19.54	3.24
Nav	<i>Navicula pelliculosa</i>	Cultivated	2.71	1.41	6.28	10.39	0.28
Nit	<i>Nitzschia palea</i>	Cultivated	3.20	0.67	6.01	9.88	0.38
Pin	<i>Pinnularia spec.</i>	Cultivated	6.84	1.13	8.91	16.87	0.54
Pin6315	<i>Pinnularia spec.</i>	Cultivated	3.35	0.00	4.52	7.87	0.59
Skel	<i>Skeletonema subsalsum</i>	Cultivated	3.96	1.35	5.63	10.94	0.45
Skel6315	<i>Skeletonema subsalsum</i>	Cultivated	5.27	2.21	5.73	13.21	0.52
Step	<i>Stephano discusminutulus</i>	Cultivated	0.78	0.62	3.87	5.27	0.14
			0.62	0.53	1.64	2.78	0.22
			1.76	1.33	6.21	9.30	0.18

investigating the chain length of *n*-alkanes, *n*-alkanoic acids and *n*-alkanols (Rieley et al., 1991; Bourbonniere and Meyers, 1996; Ficken et al., 1998, 2000). Furthermore, particularly in the case of marine environments, the branched vs. isoprenoid tetraether (BIT) index (Hopmans et al., 2004) has become a frequently used tool for assessing fluvial terrestrial OM input (Schouten et al., 2013). High BIT values (>0.8) are commonly interpreted to reflect strong soil OM input, whereas low values are interpreted to reflect a weaker soil OM imprint. However, it is increasingly reported that soils and eolian sediments, especially under dry climatic conditions, can have very low BIT values (Dirghangi et al., 2013; Zech et al., 2013b). Thus, low BIT values originating from terrestrial soils may be misinterpreted in terms of aquatic/marine origin. This short introduction and overview reveals that every terrestrial vs. aquatic proxy approach has advantages, but also disadvantages and limitations. Therefore, it seems advisable to address the question of terrestrial vs. aquatic sedimentary OM with multi-proxy approaches.

In previous studies we found that the hemicellulose-derived neutral sugars arabinose (ara) and xylose (xyl) strongly predominate over fucose (fuc) in terrestrial soils and sediments (e.g. Zech et al., 2013a). By contrast, we found fuc to occur in similar abundance to ara and xyl in lacustrine sediments (Zech et al., 2014b; Hepp et al., 2015). We have therefore investigated and reviewed here neutral sugar patterns of various terrestrial and aquatic plants, including mosses as well as algal species. We aimed to answer the question whether the sugar biomarker patterns and particularly sugar biomarker ratios, such as for instance fuc/(ara + xyl), may serve as proxies for terrestrial vs. aquatic origin of sedimentary OM input.

2. Material and methods

2.1. Sample description

The terrestrial and aquatic plant and algal samples were collected from different sites/lakes. These are (Table 1) Lake Bichlersee in the Valley Inn [47°40'33.58"N; 12°7'19.50"E; 961 m above sea level (a.s.l.)] Lake Hofstätter See about 9 km northeast of Rosenheim (47°54'4.82"N; 12°10'26.94"E; 483 m a.s.l.), a pond near Rosenheim (47°51'36.26"N; 12°8'35.58"E; 470 m a.s.l.), Lake Gemündener Maar and Lake Holzmaar in the Western Eifel (50°10'39.85"N; 6°50'12.91"E; 406 m a.s.l. and 50°7'3.10"N; 6°52'42.31"E; 430 m a.s.l., respectively) and Lake Panch Pokhari in the Helambu Himal, Nepal (28°2'30.90"N; 85°43'4.01"E; 4050 m a.s.l.).

Additionally, eleven common freshwater green algae and diatoms were cultivated and harvested in the laboratory of the Chair of Animal Ecology I, University of Bayreuth. All were obtained from the SAG Culture Collection of Algae at Göttingen University. The green algae *Nannochloropsis limnetica* (SAG 18.99), *Pediastrum boryanum* (SAG 85.81) were cultivated on Basal Medium, *Botryococcus braunii* (SAG 807-1) was cultured on MIEB12 Medium, and *Acutodesmus obliquus* (SAG 276-3a) was cultivated on Z-Medium at a concentration of $\times 0.25$. The diatoms *Gomphonema parvulum* (SAG 1032-1), *Navicula pelliculosa* (SAG 1050-3), *Nitzschia palea* (SAG 1052-3a), *Pinnularia spec.* (SAG 2386), *Skeletonema subsalsum* (SAG 8.94) and *Stephano discusminutulus* (SAG 49.91) were cultivated on bacillariophycean medium, as was *Fragilaria crotonensis* (SAG 28.96) but with an added vitamin mix. For detailed information on the culture media, see the list of culture media of the SAG (<http://www.uni-goettingen.de/en/list-of-media-and-recipes/186449.html>). After harvesting, the algae were freeze dried (Christ BETA-RVC & ALPHA 2–4; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and stored dry until analysis.

2.2. Sugar biomarker analysis

Analysis were carried out according to Zech and Glaser (2009). This method allows sugar quantification and simultaneously $\delta^{18}\text{O}$ analysis of the sugars. In brief, myoinositol was added first as internal standard. Then, the sugars were released hydrolytically from the samples using 4 M trifluoroacetic acid (TFA) for 4 h at 105 °C as described by Amelung et al. (1996). The extracted sugars were cleaned over glass fiber filters and purified using XAD and Dowex columns. After freezing and freeze-drying, derivatization was carried out with methylboronic acid (MBA; Knapp, 1979). Prior to derivatization, 3-O-methylglucose in dry pyridine was added to the samples as a second internal standard. Measurements of the individual sugars were performed at the Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin-Luther University Halle-Wittenberg using gas chromatography–pyrolysis–isotope ratio mass spectrometry (GC–Py–IRMS) with a Trace GC 2000 gas chromatograph (Thermo Fisher Scientific, Bremen, Germany) coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) via an ^{18}O -pyrolysis reactor and a GC/TC III interface (Thermo Fisher Scientific, Bremen, Germany). The monosaccharides were quantified using the myoinositol and 3-O-methylglucose internal standards.

3. Results

The relative abundances of ara, fuc and xyl in the samples are illustrated in a ternary diagram (Fig. 1a). The terrestrial and aquatic plants, including mosses, plotted close to the ara axis, whereas the algae and diatom samples plotted farther from the ara axis. This reveals that algae contain a higher relative amount of fuc than the vascular plants and mosses. According to Table 1, the algae here were characterized by fuc/(ara + xyl) ratio values >0.10 (except for samples Ped and BS8), whereas the vascular plant and moss samples were characterized by fuc/(ara + xyl) values <0.08. This finding suggests that the fuc/(ara + xyl) ratio may serve as a proxy for algal vs. vascular plant/moss origin of sedimentary OM.

The total sugar concentration (sum of ara, xyl and fuc) for the samples ranged (Table 1) from 2.26 mg/g sample (Ped, i.e. *P. boryanum* cultivated in the laboratory) to 787.87 mg/g sample (BS1, i.e. reed from Lake Bichlersee). Median total sugar concentration for grouped samples increased in the order green algae (laboratory) < diatoms (laboratory) < submerged aquatic plants < algae (lakes) < mosses < terrestrial plants < emergent plants (Fig. 2a).

4. Discussion

Our finding that ara and xyl predominate strongly over fuc in vascular plants and mosses confirms data (Fig. 1b) from D'Souza

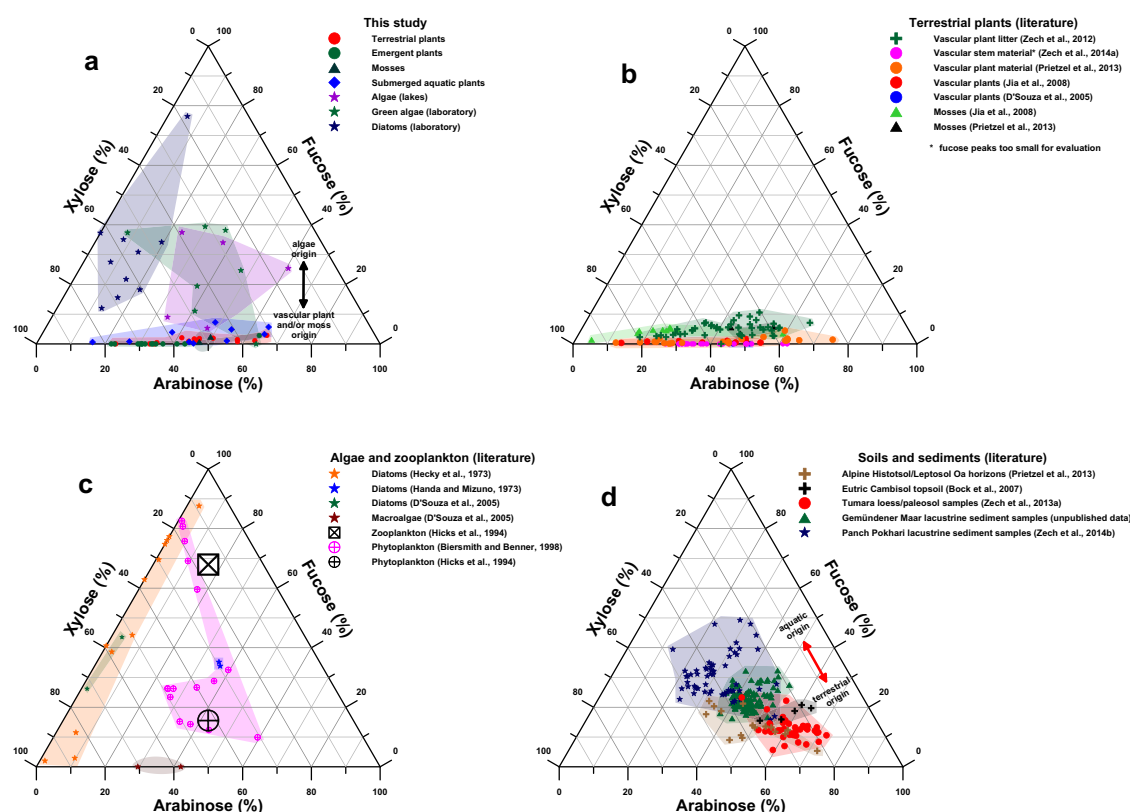


Fig. 1. Ternary diagrams depicting the relative abundance of arabinose, xylose and fucose. (a) Results from this study; (b) results of compiled terrestrial plant data from Jia et al. (2008), Zech et al. (2012, 2014a) and Prietzel et al. (2013); (c) results from a compilation of algal and zooplankton data from Handa and Mizuno (1973), Hecky et al. (1973), Hicks et al. (1994), Biersmith and Benner (1998) and D'Souza et al. (2005); (d) results from compiled soil and sediment data from Bock et al. (2007), Prietzel et al. (2013), Zech et al. (2013a, 2014b) and unpublished data.

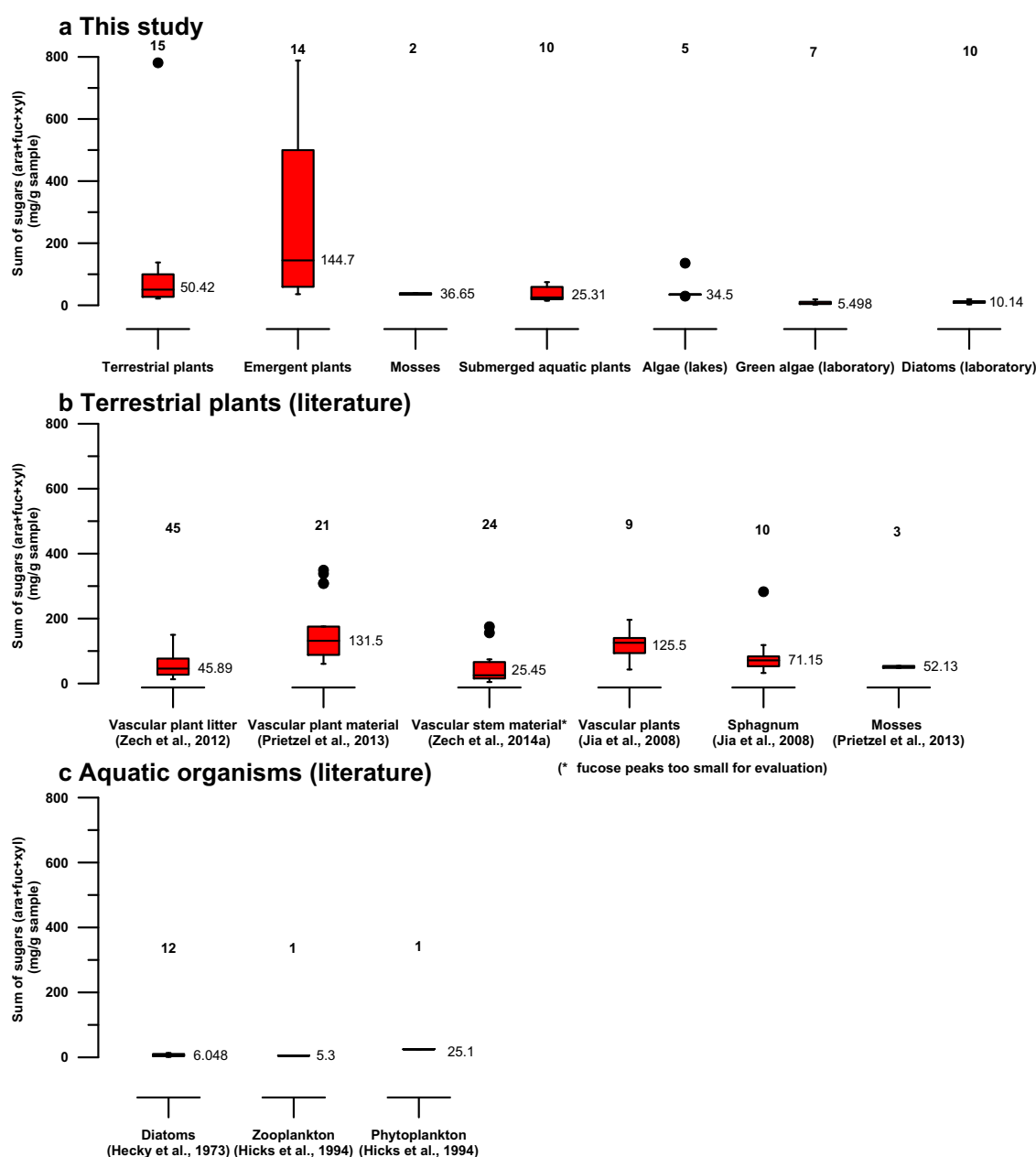


Fig. 2. Box plots of sugar concentration (sum of arabinose, fucose, and xylose). Bold numbers above the box plot represent the numbers of samples summarized in a group; numbers next to the box plot represent the median value for the group. (a) Results from own data set; (b) results from compiled terrestrial plant data from Jia et al. (2008), Zech et al. (2012, 2014a) and Prietz et al. (2013); (c) results from compiled aquatic organism data from Hecky et al. (1973) and Hicks et al. (1994).

et al. (2005), Jia et al. (2008), Zech et al. (2012, 2014a) and Prietz et al. (2013). Similarly, most of the published sugar biomarker data for algae (except for two macroalgae), as well as zooplankton (Fig. 1c), show higher relative amounts of fuc (Handa and Mizuno, 1973; Hecky et al., 1973; Hicks et al., 1994; Biersmith and Benner, 1998; D'Souza et al., 2005).

While the above compilation corroborates the proposed fuc/(ara + xyl) proxy, it may still be necessary to consider degradation

effects when interpreting sedimentary sugar biomarker results. After deposition in soils and sediments, plant- and algal-derived sugars are partly mineralized. At the same time, soil microorganisms biosynthesize sugars. Both processes may lead to post-depositional alteration of the sugar biomarker patterns of soils and sediments (Oades, 1984; Glaser et al., 2000; Ogier et al., 2001). Fig. 1d shows soil and sediment data available from the literature (Bock et al., 2007; Prietz et al., 2013; Zech et al., 2013a,

2014b) and our own unpublished data from the Lake Gemündener Maar. The Lake Panch Pokhari sugar pattern agrees well with the pattern reported by Ogier et al. (2001), measured on a sediment core from the eutrophic Lake Aydat. Accordingly, although the predominance of ara and xyl over fuc originating from plant material still exists in soils, it is slightly extenuated. These shifts could be partly explained by findings from Basler et al. (2015) indicating that ara is much more affected by microbial production than xyl. On the other hand, soil microorganisms seem to produce notable amounts of fuc (cf. review from Gunina and Kuzyakov, 2015). Nevertheless, the ternary diagram in Fig. 1d depicts that the soils can still be distinguished from lacustrine sediments on the basis of the sugar biomarker patterns.

It is noteworthy that the sediment samples from Gemündener Maar plot between the sediment samples from Lake Panch Pokhari and the soil samples. Both lakes are characterized by very small catchments (Sirocko et al., 2013; Zech et al., 2014b). However, while Lake Panch Pokhari is in a high alpine and sparsely vegetated environment and its sediment is very rich in diatoms (Krstić et al., 2012), the catchment of Gemündener Maar is densely vegetated with broadleaf trees. Hence, leaf litter input into Gemündener Maar presumably clearly dominates autochthonous OM production and explains the strong terrestrial signal for this dataset.

Our own dataset, the terrestrial plant data, as well as algae and zooplankton data from the literature (Fig. 1a–c), suggest that the ratio $\text{fuc}/(\text{ara} + \text{xyl})$ is a valuable proxy for algal OM [$\text{fuc}/(\text{ara} + \text{xyl}) > 0.10$] vs. vascular plant/moss OM [$\text{fuc}/(\text{ara} + \text{xyl}) \leq 0.10$]. Additionally, the compiled soil and sediment data (Fig. 1d) indicate that the ratio $(\text{fuc} + \text{xyl})/\text{ara}$ can help distinguish between terrestrial and aquatic sedimentary OM input. In the case of Lake Gemündener Maar, it can be concluded that fuc and xyl are primarily of aquatic origin, whereas ara is primarily of terrestrial origin. Moreover, the developed sugar biomarker ratios can help answer the question whether the sedimentary biomarkers are of autochthonous or allochthonous origin, when interpreted with compound specific $\delta^{18}\text{O}$ results.

The sugar concentration values for our own data set (Fig. 2a) are well within the range reported (Fig. 2b and c). The low sugar concentrations of lab-grown green algae and diatoms might be partly an underestimation of natural conditions. Bigogno et al. (2002) and Krienitz and Wirth (2006) found that harvesting algae in the log phase of growth, as well as specific cultural conditions, can also negatively influence algal fatty acid production. Despite limitations in the presented sugar biomarker proxies, such as different sugar concentrations characterizing different sugar sources (algal vs. terrestrial), a multi-proxy biomarker approach (suggested by e.g. Bechtel and Schubert (2009) for lake particulate organic matter) including our sugar biomarkers would provide more details on sedimentary OM sources.

5. Conclusions

The results show that the relative abundance of fucose vs. arabinose and xylose [$\text{fuc}/(\text{ara} + \text{xyl})$] is much higher in algae and zooplankton than in vascular plants and mosses. In the course of mineralization and/or degradation by soil microorganisms, the initial sugar patterns of plants may be altered. Nevertheless, the compilation of literature data suggests that lacustrine sediments and terrestrial soils can be readily distinguished using a ternary diagram with the relative abundances of fucose, arabinose and xylose. Accordingly, increased abundance of arabinose in lake sediments indicates an input of terrestrial plant material, whereas fucose and xylose are primarily of aquatic origin. We therefore, in addition to the $\text{fuc}/(\text{ara} + \text{xyl})$ ratio, propose the sugar biomarker ratio

$(\text{fuc} + \text{xyl})/\text{ara}$ as a proxy for aquatic vs. terrestrial origin of sedimentary OM in paleolimnological studies. Ideally, both sugar ratio proxies should be used within multi-proxy approaches.

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How dry was the Younger Dryas? Evidence from a coupled $\delta^2\text{H}$ – $\delta^{18}\text{O}$ biomarker paleohygrometer applied to the Gemündener Maar sediments, Western Eifel, Germany

Johannes Hepp^{1,2,a}, Lorenz Wüthrich³, Tobias Bromm², Marcel Bliedtner^{3,b}, Imke Kathrin Schäfer³, Bruno Glaser², Kazimierz Rozanski⁴, Frank Sirocko⁵, Roland Zech^{3,b}, and Michael Zech^{2,6,c}

¹Department of Geomorphology and BayCEER, University of Bayreuth, Universitätsstrasse 30, 95440 Bayreuth, Germany

²Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin Luther University Halle-Wittenberg, Von-Seckendorff-Platz 3, 06120 Halle, Germany

³Institute of Geography and Oeschger Center for Climate Change Research, University of Bern, Hallerstrasse 12, 3012 Bern, Switzerland

⁴Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Al. Mickiewicza 30, 30-059 Kraków, Poland

⁵Institute of Geosciences, Group of Climate and Sediments, Johannes Gutenberg University of Mainz, J.-J.-Becher-Weg 21, 55128 Mainz, Germany

⁶Institute of Geography, Dresden University of Technology, Helmholtzstrasse 10, 01062 Dresden, Germany

^apresent address: Department of Geomorphology and BayCEER, University of Bayreuth, Universitätsstrasse 30, 95440 Bayreuth, Germany

^bpresent address: Institute of Geography, Department of Physical Geography, Friedrich Schiller University of Jena, Löbdergraben 32, 07743 Jena, Germany

^cpresent address: Institute of Geography, Dresden University of Technology, Helmholtzstrasse 10, 01062 Dresden, Germany

Correspondence: Johannes Hepp (johannes-hepp@gmx.de)

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Abstract. Causes of the Late Glacial to Early Holocene transition phase and particularly the Younger Dryas period, i.e. the major last cold spell in central Europe during the Late Glacial, are considered to be keys for understanding rapid natural climate change in the past. The sediments from maar lakes in the Eifel, Germany, have turned out to be valuable archives for recording such paleoenvironmental changes.

For this study, we investigated a Late Glacial to Early Holocene sediment core that was retrieved from the Gemündener Maar in the Western Eifel, Germany. We analysed the hydrogen ($\delta^2\text{H}$) and oxygen ($\delta^{18}\text{O}$) stable isotope composition of leaf-wax-derived lipid biomarkers (n -alkanes C_{27} and C_{29}) and a hemicellulose-derived sugar biomarker (arabinose), respectively. Both $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ are suggested to reflect mainly leaf water of vegetation growing in the catchment of the Gemündener Maar. Leaf water re-

flects $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of precipitation (primarily temperature-dependent) modified by evapotranspirative enrichment of leaf water due to transpiration. Based on the notion that the evapotranspirative enrichment depends primarily on relative humidity (RH), we apply a previously introduced “coupled $\delta^2\text{H}_{n\text{-alkane}}$ – $\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach” to reconstruct the deuterium excess of leaf water and in turn Late Glacial–Early Holocene RH changes from our Gemündener Maar record.

Our results do not provide evidence for overall markedly dry climatic conditions having prevailed during the Younger Dryas. Rather, a two-phasing of the Younger Dryas is supported, with moderate wet conditions at the Allerød level during the first half and drier conditions during the second half of the Younger Dryas. Moreover, our results suggest that the amplitude of RH changes during the Early Holocene was

more pronounced than during the Younger Dryas. This included the occurrence of a “Preboreal Humid Phase”. One possible explanation for this unexpected finding could be that solar activity is a hitherto underestimated driver of central European RH changes in the past.

1 Introduction

In order to evaluate the relevance of man-made climate change in the future, it is of great importance to study and understand large and rapid climate fluctuations in the past. Many studies have focused on the Late Glacial to Early Holocene transition phase, a period with various expressions in temperature, atmospheric circulation and hydrology worldwide (Alley, 2000; Brauer et al., 2008; Denton et al., 2010; Partin et al., 2015; Wagner et al., 1999). Explanation for the Younger Dryas (YD) period, i.e. the major last cold spell in central Europe during the Late Glacial just before the onset of the Holocene warm period (Denton et al., 2010; Heiri et al., 2014; Isarin and Bohncke, 1999), has long been considered crucial for understanding rapid natural climate change in the past (Alley, 2000). The sediments from maar lakes in the Eifel, Germany, have turned out to be valuable archives for paleoenvironmental reconstruction by providing high-resolution palynological, sedimentological and geochemical records for climate, vegetation and landscape history (Brauer et al., 2008; Brunck et al., 2015; Litt et al., 2003; Litt and Stebich, 1999; Sirocko et al., 2013; Zolitschka, 1998).

Lacustrine sedimentary lipid biomarkers such as *n*-alkanes, originating either from leaf waxes of higher terrestrial plants (Eglinton and Hamilton, 1967) or from aquatic organisms (Volkman et al., 1998), and especially their hydrogen isotope composition ($\delta^2\text{H}_{\text{leaf-wax}/n\text{-alkane}}$), are widely accepted as paleoclimate proxies (Huang et al., 2004; Mügler et al., 2008; Sachse et al., 2004, 2012; Sauer et al., 2001). It has been demonstrated that $\delta^2\text{H}_{\text{leaf-wax}/n\text{-alkane}}$ is well correlated with the hydrogen isotope composition of precipitation ($\delta^2\text{H}_{\text{prec}}$) (e.g. Hou et al., 2008; Rao et al., 2009). Similar to the well-known ice-core and speleothem records (Alley, 2000; Luetscher et al., 2015; Rasmussen et al., 2014), lacustrine $\delta^2\text{H}_{\text{leaf-wax}/n\text{-alkane}}$ records are therefore increasingly used to reconstruct $\delta^2\text{H}$ of past precipitation and thus for deriving paleoclimatic information (cf. Araguás-Araguás et al., 2000; Dansgaard, 1964; Rozanski et al., 1993). However, the alteration of $\delta^2\text{H}_{\text{prec}}$ either through evapotranspirative ^2H enrichment of leaf or lake water can challenge a robust $\delta^2\text{H}_{\text{prec}}$ reconstruction (e.g. Mügler et al., 2008; Zech et al., 2015). Apart from $\delta^2\text{H}_{n\text{-alkane}}$, the oxygen isotope composition of hemicellulose- or polysaccharide-derived sugars ($\delta^{18}\text{O}_{\text{sugar}}$) was established as a tool in paleoclimate research during recent years (Zech et al., 2011, 2013a, 2014a). Analogous to $\delta^2\text{H}_{n\text{-alkane}}$, $\delta^{18}\text{O}_{\text{sugar}}$ is affected by the isotope composition of source water, which is closely related to the local precipi-

tation ($\delta^{18}\text{O}_{\text{prec}}$) as well as by evapotranspirative ^{18}O enrichment (Tuthorn et al., 2014; Zech et al., 2013b, 2014b). Moreover, it was suggested that the coupling of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results can help to disentangle $\delta^2\text{H}/\delta^{18}\text{O}_{\text{prec}}$ changes and variable $^2\text{H}/^{18}\text{O}_{\text{leaf/lake-water}}$ enrichment (Henderson et al., 2010; Hepp et al., 2015, 2017; Tuthorn et al., 2015; Voelker et al., 2014, 2015; Zech et al., 2013a). For instance, Voelker et al. (2014) presented a framework for using $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of tree-ring cellulose in order to infer relative air humidity (RH). Tuthorn et al. (2015) validated a previously suggested “coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach”. Accordingly, the application of that approach to an Argentinian topsoil transect yielded a highly significant correlation of actual and biomarker-based reconstructed RH values ($R = 0.79$, $p < 0.001$, $n = 20$). Both approaches were successfully applied to loess–paleosol sequences (Hepp et al., 2017; Zech et al., 2013a) and subfossil wood (Voelker et al., 2015). By contrast, the application of the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach to a lacustrine archive is still missing.

Within this study, we aimed at applying the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach to the Late Glacial–Early Holocene sediment cores of the Gemündener Maar. More specifically, we addressed the following objectives: (i) source identification of the sedimentary organic matter and the investigated *n*-alkanes and sugars (aquatic vs. terrestrial), (ii) reconstructing leaf water isotope composition based on compound-specific $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the *n*-alkane and sugar biomarkers, (iii) reconstructing RH changes using the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach, and (iv) inferring implications for central European paleoclimate history from the established Gemündener Maar RH record.

2 Material and methods

2.1 The Gemündener Maar and sampling

The Gemündener Maar is located in the Eifel volcanic fields in western Germany at an altitude of 407 m a.s.l. (50°10′39.853″ N, 6°50′12.912″ E; Fig. 1a and b; Sirocko et al., 2013). The maar was formed during a phreatomagmatic explosion within the local Devonian siltstone (greywacke) around 20–25 ka (Büchel, 1993). The lake is 39 m deep at its maximum and has a diameter of roughly 300 m. Due to its formation conditions the lake is almost circular with a lake surface area of 75 000 m² and is surrounded by a small catchment (Fig. 1b), with an area of 430 000 m² (Scharf and Menn, 1992). The lake is fed by precipitation and groundwater (no surface inflow and outflow present). The sediments are, accordingly, not affected by fluvial sediment input. The catchment area is furthermore steep and densely vegetated with broadleaved trees (Fig. 1c). The investigated samples were taken from the 8 m Gemündener Maar core (GM1), which was taken at approximately 20 m water depth near the

centre of the maar (Fig. 1b) with a Livingston piston corer (UWITEC, Mondsee, Austria). The GM1 core was retrieved from a terrace on the steep slope of the maar exactly in a fan of an underwater erosion gully structure. The core is part of the Eifel Laminated Sediment Archive Project of the Institute for Geoscience at Johannes Gutenberg University Mainz (Sirocko et al., 2013, 2016).

2.2 Bulk analysis and pollen analysis

Bulk analyses were carried out on 112 samples, covering a section of 606 to 727 cm depth of the Gemündener Maar GM1 core. Total carbon (TC) and nitrogen (N), bulk carbon isotope composition ($\delta^{13}\text{C}_{\text{TC}}$), and nitrogen isotope composition ($\delta^{15}\text{N}$) were determined at the Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin Luther University Halle-Wittenberg, using EuroVector EA 3000 elemental analyser (Hekatech, Wegberg, Germany) coupled via a ConFlo III Interface to a Delta V Advantage isotope ratio mass spectrometer (IRMS; both from Thermo Fisher Scientific, Bremen, Germany). Additionally, total organic carbon (TOC) and bulk $\delta^{13}\text{C}$ of the total organic carbon ($\delta^{13}\text{C}_{\text{TOC}}$) were assessed after removal of carbonate with 32 % hydrochloric acid (HCl) fumigation followed by a neutralisation step with moist sodium hydroxide, both for 24 h under 60 °C and vacuum conditions. This allows calculating TOC/N atomic ratios. Laboratory standards from the International Atomic Energy Agency (IAEA) as well as from the United States Geological Survey (USGS) with known total carbon, nitrogen, ^{13}C and ^{15}N contents (IAEA N2, IAEA CH6, IAEA NO3, IAEA CH7, IAEA 305A, USGS 41) were used for calibration. The ^{13}C and ^{15}N contents are expressed in the common δ notation as relative to an international standard ($\delta^{13}\text{C}$: Vienna Pee Dee Belemnite, VPDB; $\delta^{15}\text{N}$: atmospheric N_2 , air).

For pollen analysis, 16 samples were investigated covering the relevant depth section. Each sample covered a depth range of 1 cm. Preparation was conducted by Frank Dreher according to standard procedures at the laboratory of the Group of Climate and Sediments, Institute of Geosciences, Johannes Gutenberg University of Mainz, using potassium hydroxide, HCl and hydrofluoric acid (Sirocko et al., 2016). Afterwards, acetic acid and a mixture of acetic anhydride and sulfuric acid (9 : 1) were used for acetolysis. The samples were then centrifuged at 3000 to 3500 rpm for 5 min and then sieved over a 200 and a 10 μm sieve. Afterwards, the samples were fixed with anhydrous glycerol for reliable identification, and a maximum magnification of 600 was used for counting the remains. Pollen results are reported in relative percentages (%).

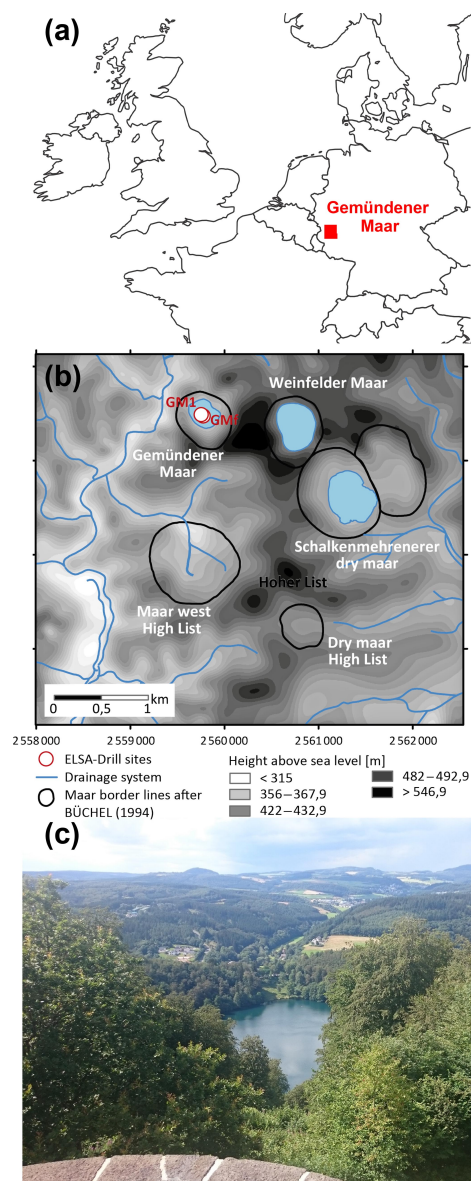


Figure 1. (a) Location of the Gemündener Maar in the Eifel region in Germany (generated using OpenStreetMap homepage, ©OpenStreetMap contributors, <https://www.openstreetmap.org>, last access: 15 August 2016). (b) Digital terrain model and drainage system of the immediate surroundings of the Gemündener Maar, with maar borders according to Büchel (1994) representing the size of the crater. In addition, the core position is displayed (GM1; 50°10′39.853″ N, 6°50′12.912″ E) along with the short core named GMf (not part of this study) marked as ELSA drill sites. Both cores are part of the Eifel Laminated Sediment Archive Project (ELSA project). (c) Photo of Gemündener Maar showing the steep and densely forested catchment (by Michael Zech, reproduced with his permission).

2.3 Age control

The investigated sediments are partially laminated. The first tie point to establish a chronology for the Gemündener Maar core is a radiocarbon-dated piece of charcoal in 727 cm core depth, which dates to $13\,800 \pm 110$ a cal BP (Fig. 2d). This age is derived from a ^{14}C age of $11\,950 \pm 65$ a BP as part of the Supplement of Sirocko et al. (2013), calibrated using CalPal software (Weninger and Jöris, 2008) calculated with the IntCal13 calibration curve (Reimer et al., 2013). The uncertainty of the calibrated ^{14}C age represents the 68 % probability range. The second tie point is the clearly visible Laacher See Tephra between 673 and 680 cm core depth (Fig. 2a and d). The latter can be used as chronological marker due to the varve-counted age of 12 880 a BP in the adjacent Meerfelder Maar (Brauer et al., 1999). The onset of the Younger Dryas period was set to 12 680 a BP (varve-counted in Meerfelder Maar sediments; Brauer et al., 1999; Litt et al., 2009) identified at a depth of 670 cm in the GM1 core due to a clear colour change (Fig. 2a and d). The onset of the Preboreal (Holocene) was found to date to 11 590 a BP in Meerfelder Maar by varve counting (Litt et al., 2009). This was used to wiggle-match the distinct changes in the pollen spectra (decreasing *Poaceae*, peaking *Artemisia*, increasing *Pinus* and *Betula*; Fig. 2b and c), the clear rise in TOC (Fig. 3a) and the colour change (Fig. 2a), which were identified at 643 cm depth (Fig. 2d). The Late Glacial to Preboreal (Holocene) transition is commonly well recorded in maar sediments from the Eifel region, i.e. clear changes in deposition as well as pollen pattern (Brauer et al., 1999; Litt et al., 2001, 2003; Litt and Stebich, 1999), dated to 11 600 a BP, e.g. in Holzmaar, by a combination of varve counting and ^{14}C dating (Zolitschka, 1998). The last time marker used to constrain the age model is the middle of the sharp increase in *Corylus* (hazel) pollen at 622 cm depth (Fig. 2b, c and d). We used this sharp increase as a marker for the Preboreal to Boreal transition, which is varve-counted by Litt et al. (2009) to 10 740 a BP in the Meerfelder Maar sediments. The offset of 60 years to the varve-counted Holzmaar record of Zolitschka (1998), as it is presented by Litt et al. (2009), is within the uncertainty of placing the onset of the Preboreal in the Gemündener Maar *Corylus* curve.

The investigated core section from 607 to 694 cm depth therefore covers the time between $\sim 13\,150$ and $10\,140$ a BP, i.e. the Allerød, the Younger Dryas, the Preboreal, and the beginning of the Boreal, with regard to the biomarkers (Fig. 2a and d). Assuming constant sedimentation rates between the markers, an average resolution of 51 a cm^{-1} can be calculated; the minimum and maximum resolution are 19 and 124 a cm^{-1} , respectively. The part above the Laacher See Tephra reveals a lower mean resolution (55 a cm^{-1}) than the section below (30 a cm^{-1}).

2.4 Biomarker and compound-specific isotope analysis

For $\delta^2\text{H}$ analyses of *n*-alkanes as well as $\delta^{18}\text{O}$ analyses of sugars, 59 samples were prepared from 607 to 694 cm depth of the Gemündener Maar GM1 core, in order to cover the core section with already high TOC content and the Late Glacial to Holocene transition (Figs. 2 and 3a). *n*-Alkanes were extracted from 1 to 6 g freeze-dried and ground samples by microwave extraction at 100°C for 1 h, using 15 mL of solvent (dichloromethane and methanol, at a ratio of 9 : 1). The resultant total lipid extracts were separated over aminopropyl silica gel (Supelco $45\text{ }\mu\text{m}$) filled pipette columns. Non-polar compounds (including *n*-alkanes) were eluted with *n*-hexane. The fraction was spiked with a known amount of 5α -androstanone, used as an internal standard. Identification and quantification was carried out on an Agilent MS 5975 (EI) interfaced with an Agilent 7890 GC equipped with a 30 m fused silica capillary column (HP5-MS 0.25 mm i.d. , $0.25\text{ }\mu\text{m}$ film thickness) and a split-splitless injector operating in splitless mode at 320°C . Carrier gas was helium and the temperature program was 1 min at 50°C , from 50 to 200°C at $30^\circ\text{C min}^{-1}$, from 200 to 320°C at 7°C min^{-1} , and 5 min at 320°C . Data recording comprised the total ion count (scan mode from m/z 40 to 600) and single-ion monitoring (m/z 57, 71, 85 and 99). Concentrations were calculated relative to the internal standard and to an external standard (*n*-C₂₁ to *n*-C₄₀ alkane mixture, Supelco), injected in different concentrations (40, 4, 1, $0.4\text{ ng }\mu\text{L}^{-1}$).

Prior to compound-specific isotope analyses, the *n*-alkanes were further purified. The non-polar fractions were passed over a pipette column filled with activated AgNO_3 impregnated silica gel and a pipette column filled with zeolite (Geokleen). After drying, the zeolite was removed using hydrofluoric acid and the *n*-alkanes were recovered by liquid-liquid extraction with hexane. The purified *n*-alkane fractions were measured for their compound-specific stable hydrogen isotope composition ($\delta^2\text{H}$). The measurements were performed at the Institute of Geography, University of Bern on an IsoPrime 100 IRMS, coupled to an Agilent 7890A GC via a GC5 pyrolysis or combustion interface operating in pyrolysis modus with a Cr (ChromeHD) reactor at 1000°C . Samples were injected with a split-splitless injector. The GC was equipped with 30 m fused silica column (HP5-MS, 0.32 mm inner diameter, $0.25\text{ }\mu\text{m}$ film thickness). The precision was checked by co-analysing a standard alkane mixture (*n*-C₂₇, *n*-C₂₉, *n*-C₃₃) with known isotope composition (Arndt Schimmelmann, University of Indiana), injected twice every six runs. The samples were analysed in three repetitions (except from the samples in 622 and 672 cm depth), and the analytical precision was generally better than 5 ‰. The stable hydrogen isotope compositions are given in the δ notation ($\delta^2\text{H}_{n\text{-alkane}}$) versus Vienna Standard Mean Ocean Water (VSMOW). The H_3^+ -correction factor was checked every 2 days and stayed stable over the course of measurements at 3.14. The $\delta^2\text{H}_{n\text{-alkane}}$ values refer to the area-weighted

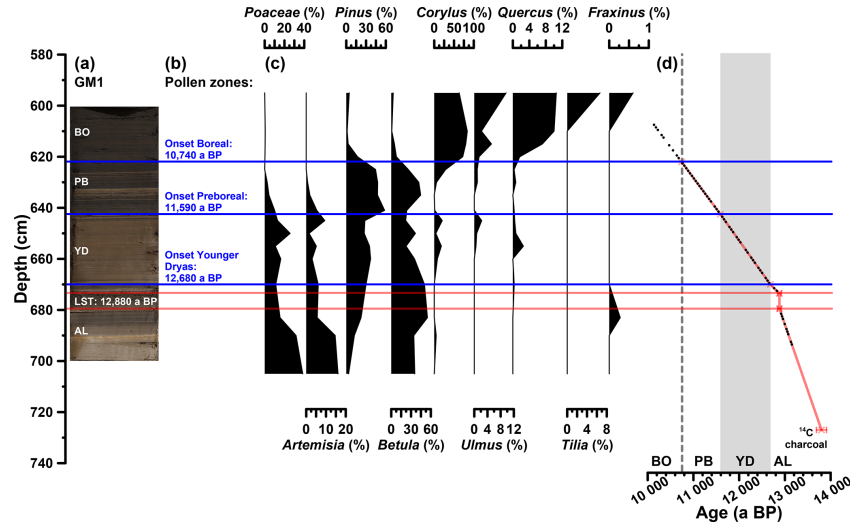


Figure 2. (a) Photo of the investigated GM1 core section, with regard to the biomarkers (607 to 694 cm depth), displaying the position of the Laacher See Tephra (LST), varve-counted to 12 880 a BP in the adjacent Meerfelder Maar (cf. Brauer et al., 1999). (b) Defined pollen zones according to Brauer et al. (1999) and Litt et al. (2009). (c) Pollen profiles of pollen groups, which were used for defining the pollen zones. Pollen analysis was carried out by Frank Dreher (Johannes Gutenberg University of Mainz). (d) Age–depth model of the full investigated GM1 section (606 to 727 cm depth) consisting of a ^{14}C -dated piece of charcoal, the LST and the onsets of the Younger Dryas, Preboreal and Boreal (Holocene). Additionally, the biomarker sampling points are displayed (black points). The error bars of the ^{14}C age and the LST represent the uncertainty of the calibration (68 % probability range) and the error during of the varve counting (± 40 a; Brauer et al., 1999), respectively.

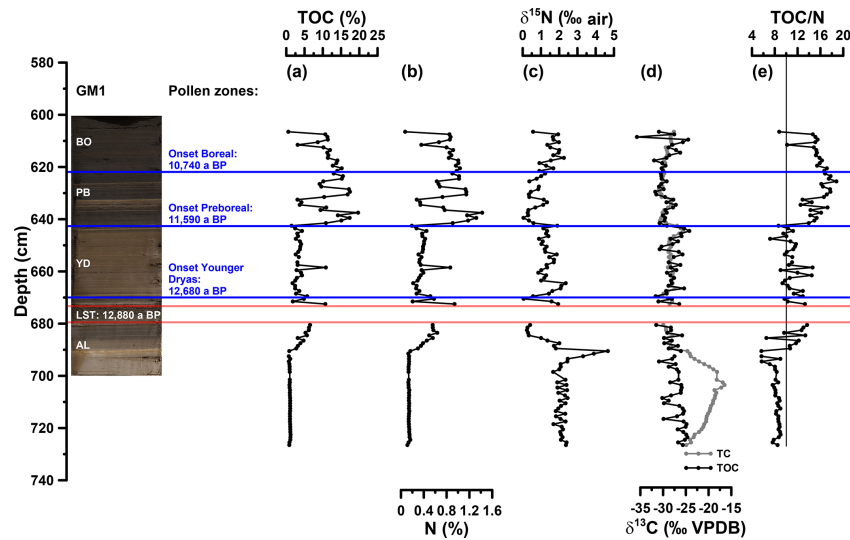


Figure 3. (a) Depth profiles of total organic carbon (TOC), (b) total nitrogen (N), (c) bulk stable nitrogen isotope composition ($\delta^{15}\text{N}$), (d) stable carbon isotope composition of total carbon (TC) and TOC ($\delta^{13}\text{C}_{\text{TC}}$, $\delta^{13}\text{C}_{\text{TOC}}$) and (e) carbon to nitrogen atomic ratio (TOC/N). The vertical line in (e) indicates a TOC/N atomic ratio threshold of 10 (Meyers, 2003). AL: Allerød; YD: Younger Dryas; PB: Preboreal; BO: Boreal.

mean of the $\delta^2\text{H}$ values of n -alkanes with 27 and 29 carbon atoms ($n\text{-C}_{27}$, $n\text{-C}_{29}$) because of their relatively high abundance in the samples (Fig. 4a).

The sample preparation for $\delta^{18}\text{O}$ analyses of hemicellulose- or polysaccharide-derived sugars followed standard procedures at the Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin Luther University Halle-Wittenberg, according to the method of Zech and Glaser (2009). The monosaccharide sugars were hydrolytically extracted from samples containing approximately 10 mg total organic carbon with 10 mL of 4 M trifluoroacetic acid at 105 °C for 4 h, applying the method described by Amelung et al. (1996). After filtration over glass fibre filters, the extracted sugars were cleaned using XAD-7 (to remove humic-like substances) and Dowex 50WX8 columns (to remove interfering cations). Afterwards, the purified samples were freeze-dried and derivatised by adding methylboronic acid (4 mg in 400 μL pyridine) for 1 h at 60 °C.

The compound-specific $\delta^{18}\text{O}$ measurements were performed using a Trace GC 2000 coupled to a Delta V Advantage IRMS via an ^{18}O -pyrolysis reactor (GC IsoLink) and a ConFlo IV interface (all devices from Thermo Fisher Scientific, Bremen, Germany). Each sample was measured in threefold repetition, embedded in-between co-derivatised sugar standards at various concentrations and known $\delta^{18}\text{O}$ values. The $\delta^{18}\text{O}$ values of the samples are expressed in δ notation ($\delta^{18}\text{O}_{\text{sugar}}$) versus VSMOW. The measured $\delta^{18}\text{O}_{\text{sugar}}$ values were corrected for drift, amount and area dependency and also for the hydrolytically introduced oxygen atoms that form carbonyl groups with the C1 atoms of the sugar molecules (Zech and Glaser, 2009). Mean standard errors for the triplicate measurements of all 59 samples are 0.6‰, 0.7‰ and 0.7‰ for arabinose, fucose and xylose, respectively. The $\delta^{18}\text{O}_{\text{sugar}}$ values refer to the $\delta^{18}\text{O}$ values of the monosaccharides arabinose, fucose and xylose (Fig. 4b). Rhamnose areas, or concentrations, were too low for reliable isotope measurements in most samples.

2.5 Conceptual framework of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{--}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach

The coupled $\delta^2\text{H}_{n\text{-alkane}}\text{--}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach was described in detail by Tuthorn et al. (2015) and Zech et al. (2013a). The most fundamental assumption of the approach is that the isotope composition of leaf water can be reconstructed by applying biosynthetic fractionation factors on the measured $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ values (Fig. 5). The concept is furthermore based on the observation that the isotope composition of global precipitation plots typically close to the global meteoric water line (GMWL; $\delta^2\text{H}_{\text{prec}} = 8 \cdot \delta^{18}\text{O}_{\text{prec}} + 10$; Dansgaard, 1964). In Germany, a local meteoric water line (LMWL_{Germany}) slightly deviating from GMWL was described by Stumpp et al. (2014) ($\delta^2\text{H}_{\text{prec}} = 7.72 \pm 0.13 \cdot \delta^{18}\text{O}_{\text{prec}} + 4.90 \pm 0.01$; Fig. 5), which we used as

the baseline for our calculations. The quite similar LMWLs for Trier ($\delta^2\text{H}_{\text{prec}} = 7.81 \pm 0.08 \cdot \delta^{18}\text{O}_{\text{prec}} + 5.06 \pm 0.60$) and Koblenz ($\delta^2\text{H}_{\text{prec}} = 7.80 \pm 0.07 \cdot \delta^{18}\text{O}_{\text{prec}} + 2.68 \pm 0.53$) as well as the GMWL are additionally displayed in Fig. 5 for comparison. The local precipitation is the source for soil water and shallow groundwater, which in turn acts as source water for plants. During daytime, however, leaf water is typically ^2H - and ^{18}O -enriched compared to the source water due to evapotranspiration through the stomata (Fig. 5; Allison et al., 1985; Bariac et al., 1994; Walker and Brunel, 1990). The leaf water reservoir at the evaporative sites quickly achieves steady-state conditions (Allison et al., 1985; Bariac et al., 1994; Gat et al., 2007; Walker and Brunel, 1990). Thus, the isotope composition of the transpired water vapour is equal to the isotope composition of the source water utilised by the plants during the transpiration process. The evaporative enrichment of leaf water under steady-state conditions can be described via a Craig–Gordon model (e.g. Flanagan et al., 1991; Roden and Ehleringer, 1999) by the following expression (e.g. Barbour et al., 2004):

$$\delta_e \approx \delta_s + \varepsilon^* + \varepsilon_k + (\delta_a - \delta_s - \varepsilon_k) \frac{e_a}{e_i}, \quad (1)$$

where δ_e , δ_s and δ_a are the hydrogen and oxygen isotope compositions of leaf water at the evaporative sites, in source water and in atmospheric water vapour, respectively; ε^* is the equilibrium enrichment expressed as $(1 - 1/\alpha_{L/V}) \times 10^3$ where $\alpha_{L/V}$ is the equilibrium fractionation between liquid and vapour in ‰; and ε_k is the kinetic fractionation parameters for water vapour diffusion from intracellular air space through the stomata and the boundary layer, both for ^2H and ^{18}O , respectively; and e_a/e_i is the ratio of atmospheric vapour pressure to intracellular vapour pressure. When leaf temperature is equal to air temperature, the e_a/e_i ratio represents the RH of the local atmosphere. If the plant source water and the local atmospheric water vapour are in isotopic equilibrium, the term $\delta_a - \delta_s$ can be approximated by $-\varepsilon^*$. Thus, Eq. (1) can be reduced to

$$\delta_e \approx \delta_s + (\varepsilon^* + \varepsilon_k) (1 - \text{RH}). \quad (2)$$

The kinetic fractionation parameters (ε_k) are typically related to stomatal and boundary layer resistances with respect to water flux (Farquhar et al., 1989). Since direct measurements of those plant physiological parameters can be hardly assessed in a paleo application, we used the kinetic enrichment parameters C_k instead, derived from a more generalised form of the Craig–Gordon model, for describing the kinetic isotope enrichment for ^2H and ^{18}O , which leads to Eq. (3) (Craig and Gordon, 1965; Gat and Bowser, 1991):

$$\delta_e \approx \delta_s + (\varepsilon^* + C_k) (1 - \text{RH}). \quad (3)$$

In a $\delta^2\text{H}\text{--}\delta^{18}\text{O}$ diagram, the hydrogen and oxygen isotope composition of leaf and source water can be described as a local deuterium (d) excess = $\delta^2\text{H} - 7.72 \cdot \delta^{18}\text{O}$ (Stumpp

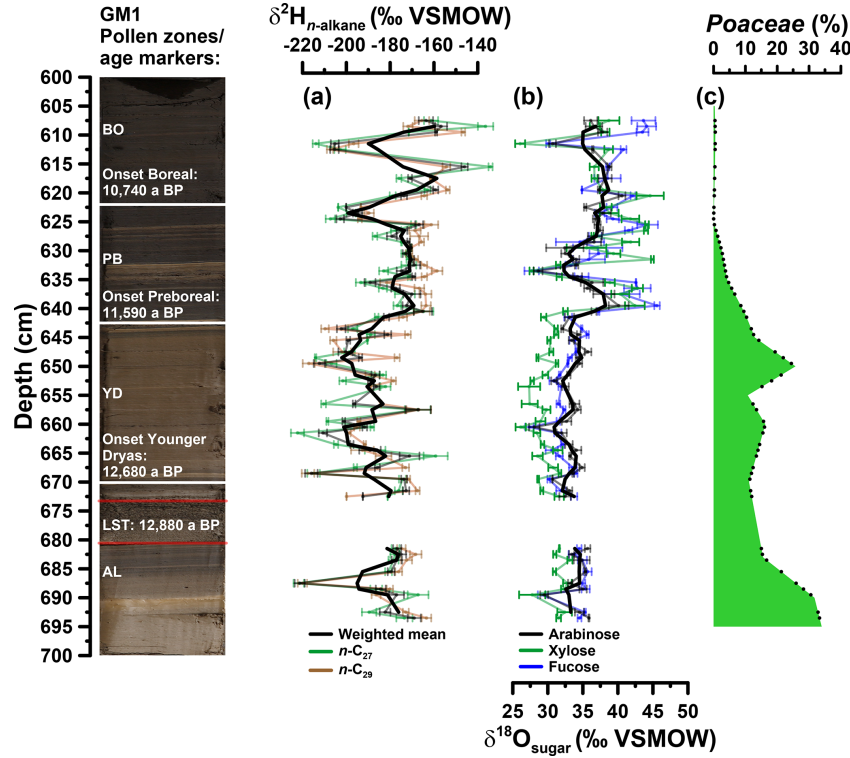


Figure 4. (a) Depth profiles of compound-specific stable hydrogen isotope composition of the individual alkanes $n\text{-C}_{27}$ and $n\text{-C}_{29}$ and the weighted mean ($\delta^2\text{H}_{n\text{-alkane}}$). (b) Compound-specific stable oxygen composition of the individual sugars arabinose, xylose and fucose ($\delta^{18}\text{O}_{\text{sugar}}$). Error bars show analytical standard errors; bold lines show three-point moving averages. (c) Depth profile of *Poaceae* pollen. Additionally, the resampled data points (black points) used for the grass correction procedures (Eqs. 10 and 11) are displayed. In addition, the GM1 core picture with the age markers used is displayed. AL: Allerød; LST: Laacher See Tephra; YD: Younger Dryas; PB: Preboreal; BO: Boreal.

et al., 2014) in one equation by using the slope of the LMWL_{Germany} (Eq. 4). This approach is comparable to the d excess definition from Dansgaard (1964), who used the equation $d = \delta^2\text{H} - 8 \cdot \delta^{18}\text{O}$ for a measure of the parallel deviation between a given point in the $\delta^2\text{H}$ – $\delta^{18}\text{O}$ diagram from the GMWL.

$$d_e \approx d_s + \left(\varepsilon_2^* - 7.72 \cdot \varepsilon_{18}^* + C_k^2 - 7.72 \cdot C_k^{18} \right) (1 - \text{RH}), \quad (4)$$

where d_e and d_s are the d excess values of the leaf water at the evaporative sites and the source water, respectively, and the equilibrium (ε_2^* and ε_{18}^*) and kinetic enrichment parameters (C_k^2 and C_k^{18}) are expressed for both isotopes. From Eqs. (1) to (4) the primary control of RH on the isotope composition of the leaf water is demonstrated when stomata are open through transpiration. If d_e can be derived from compound-specific $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurements of the n -alkane and sugar biomarkers, which derive $\delta^2\text{H}_e$ and $\delta^{18}\text{O}_e$ values for the purpose of calculating d_e values via the equa-

tion $d_e = \delta^2\text{H}_e - 7.72 \cdot \delta^{18}\text{O}_e$, the d_s can also be approximated from the d excess of the LMWL_{Germany} (= 4.9). Accordingly, Eq. (4) can be rearranged in order to calculate the RH of the local atmosphere normalised to leaf temperature as given by Eq. (5) (Hepp et al., 2017; Tuthorn et al., 2015; Zech et al., 2013a):

$$\text{RH} \approx 1 - \frac{\Delta d}{(\varepsilon_2^* - 7.72 \cdot \varepsilon_{18}^* + C_k^2 - 7.72 \cdot C_k^{18})}, \quad (5)$$

where Δd is the distance between d_e and d_s , calculated as $\Delta d = d_e - d_s$. Equilibrium fractionation parameters (ε_2^* and ε_{18}^*) are derived from empirical equations of Horita and Wesolowski (1994), with mean daytime growth-period temperature of 14.8 °C (from 06:00 to 19:00 CET and April to October, derived from the Nürburg-Barweiler station, approx. 25 km northeast of Gemündener Maar; hourly data from 1995 to 2015 from Deutscher Wetterdienst, 2016). Equilibrium fractionation factors equal 83.8 ‰ and 10.15 ‰ for ^2H and ^{18}O , respectively. The kinetic fractionation pa-

rameters (C_k^{22} and C_k^{18}) for ^2H and ^{18}O are set to 25.1 ‰ and 28.5 ‰, respectively, according to Merlivat (1978), who reported maximum values during the molecular diffusion process of water through a stagnant boundary layer. The assumption that maximum kinetic fractionation occurs seems to be most suitable for sedimentological application where a signal averaging over decades can be assumed (see above and discussion in Zech et al., 2013a). It should also be noted that ε_k values of broadleaf trees and shrubs over broad climatic conditions are well within the range of the C_k^{22} and C_k^{18} values used, revealing 23.9 ‰ (± 0.9) and 26.7 ‰ (± 1.0) for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively (derived from the Supplement of Cernusak et al., 2016).

The numerator of Eq. (5) describes the parallel distance between the d excesses of LMWL and leaf water at the evaporative sites, which is converted into RH values, while the denominator is a combination of the slopes of LMWL and the local evaporation line (LEL). This means in turn that the quantification with Eq. (5) is done by obtaining the distance between the source water points, calculated via the intersects between the individual LELs and the LMWL_{Germany}, and the leaf water points. The underlying slope of those LELs can be derived from Eq. (6) via the Craig–Gordon model using the same assumptions as outlined above in a rearranged form (Eq. 6; Zech et al., 2013a). When using the fractionation parameters from above, the slope of the LEL is constant over time, independent of RH and equal to ~ 2.8 (Eq. 6). This agrees well with field and laboratory studies (Allison et al., 1985; Bariac et al., 1994; Gat et al., 2007; Tipple et al., 2013; Walker and Brunel, 1990).

$$S_{\text{LEL}} = \frac{\delta_e^2 - \delta_s^2}{\delta_e^{18} - \delta_s^{18}} \approx \frac{\varepsilon_2^* + C_k^{22}}{\varepsilon_{18}^* + C_k^{18}} \quad (6)$$

In order to provide an uncertainty interval in terms of measurement precision covering the Gemündener Maar RH record, we calculated an error propagation for d_e values according to Eq. (7), by using the analytical standard errors (SEs). Maximum and minimum values were then applied to Eq. (5) resulting in a lower and upper RH limit (blue-shaded area in Fig. 7a).

$$\text{SE}_{d_e} = \sqrt{(\text{SE}_{\delta^2\text{H}_{n\text{-alkane}}})^2 + 7.72 \cdot (\text{SE}_{\delta^{18}\text{O}_{\text{sugar}}})^2} \quad (7)$$

3 Results and discussion

3.1 Source identification of bulk organic matter and of the investigated n -alkane and sugar biomarkers

For basic sedimentological characterisation, TOC, N, $\delta^{15}\text{N}$, $\delta^{13}\text{C}_{\text{TC}}$ and $\delta^{13}\text{C}_{\text{TOC}}$ values as well as the TOC/N atomic ratios (Fig. 3a to e) are displayed from 605 to 727 cm depth. TOC values range from 0.6 % to 19.7 %. N ranges from 0.1 % to 1.4 % and correlates highly significantly with TOC ($r = 0.99$, $p < 0.001$, $n = 110$). Higher TOC contents during

the Allerød, Preboreal and Boreal likely reflect warmer conditions being favourable for terrestrial and aquatic biomass production, whereas lower TOC values during the Younger Dryas likely reflect less favourable conditions for biomass production and possibly increasing minerogenic sedimentation. Interestingly, the Late Glacial–Early Holocene TOC patterns seem not to be the same for all maar lakes, because the Meerfelder Maar shows a clear TOC two-phasing during the Younger Dryas (Brauer et al., 1999) and the Holzmaar is lacking an Allerød TOC maximum (Lücke et al., 2003). The $\delta^{15}\text{N}$ values of the Gemündener Maar record range from 0 ‰ to 5 ‰, showing the maximum and minimum within the Allerød period. $\delta^{13}\text{C}_{\text{TC}}$ and $\delta^{13}\text{C}_{\text{TOC}}$ reveal values between -31 ‰ and -17 ‰ and -36 ‰ and -24 ‰, respectively. While $\delta^{13}\text{C}_{\text{TC}}$ shows maximum values at 703 cm depth, $\delta^{13}\text{C}_{\text{TOC}}$ is decreasing continuously from the beginning to the end of the Allerød, followed by increasing values during the Younger Dryas and the Preboreal and Boreal, interrupted by a short decrease around the beginning of the Holocene. $\delta^{13}\text{C}_{\text{TC}}$ clearly shows the presence of carbonate between 690 and 727 cm depth with less negative $\delta^{13}\text{C}_{\text{TC}}$ values compared to $\delta^{13}\text{C}_{\text{TOC}}$ values. TOC/N atomic ratios range from 6 to 19 with the end of the Allerød revealing increasing ratios, while the late Younger Dryas shows slightly decreasing ratios and the Preboreal is marked by the highest ratios.

The source of organic matter in lacustrine sediments of small lakes, as one of the most crucial questions and challenges when interpreting organic proxies from lacustrine sedimentary records (Meyers and Ishiwatari, 1993), can either be autochthonous (aquatic origin) or allochthonous (terrestrial origin). The TOC/N ratio and $\delta^{13}\text{C}$ values are the most common proxies for sedimentary source determination. While non-vascular aquatic organisms often reveal C/N atomic ratios between 4 and 10 (due to low amounts of cellulose and lignin), vascular plants commonly show C/N atomic ratios of 20 and higher (Meyers and Ishiwatari, 1993). According to Meyers (2003), a TOC/N atomic ratio of 10 is often used as threshold for identifying aquatic versus terrestrial input (Fig. 3e). Accordingly, the input from terrestrial organic matter increased during the Allerød, decreased slightly during the Younger Dryas and was highest during the Holocene. The Gemündener Maar $\delta^{13}\text{C}_{\text{TOC}}$ values (Fig. 3d) are well within the range of C_3 land plants and lacustrine algae (Meyers and Lallier-Vergés, 1999); evidence for the occurrence of C_4 land plants is missing. Overall, no clear additional information about the sedimentary organic matter origin of the Gemündener Maar sediments can be inferred neither from $\delta^{13}\text{C}_{\text{TOC}}$ alone (cf. Lücke et al., 2003) nor by combining $\delta^{13}\text{C}_{\text{TOC}}$ with TOC/N ratios (cf. Meyers and Lallier-Vergés, 1999). When considering that both $\delta^{13}\text{C}_{\text{TOC}}$ and TOC/N values of terrestrial organic matter are additionally affected by mineralisation and degradation, resulting in more positive $\delta^{13}\text{C}_{\text{TOC}}$ values and lower TOC/N ratios (e.g. Zech et al., 2007), a straightforward interpretation of those

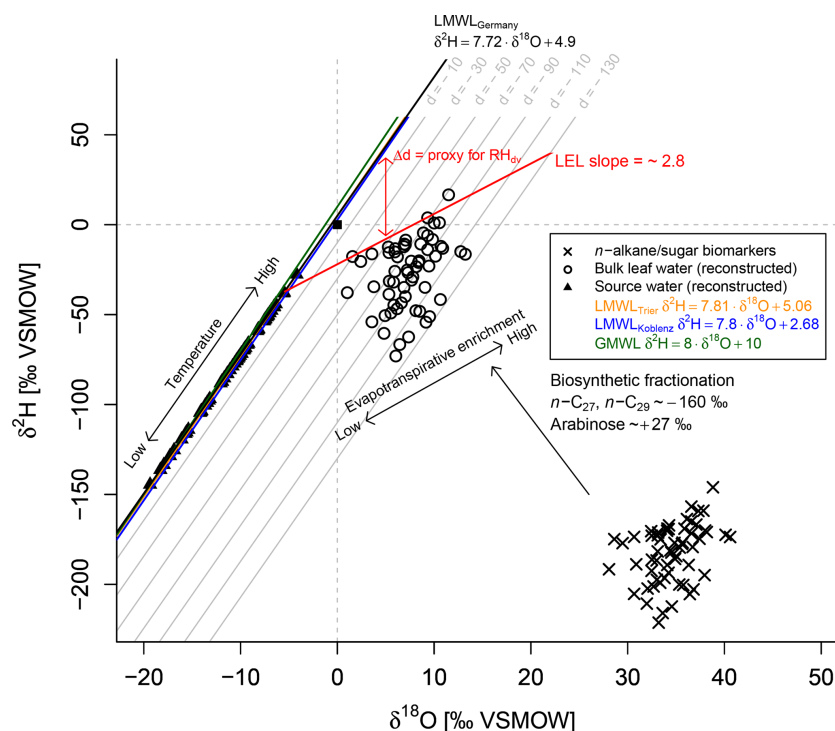


Figure 5. Conceptual framework of the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{--}\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach displayed as $\delta^{18}\text{O}\text{--}\delta^2\text{H}$ diagram showing the measured n -alkanes (weighted mean of $n\text{-C}_{27}$ and $n\text{-C}_{29}$) and sugar (arabinose) biomarkers (black crosses), the reconstructed leaf water (open circles), the global meteoric water line (GMWL, green line), and the local meteoric water lines of Germany (LMWL_{Germany}, black line), Trier (LMWL_{Trier}, yellow line) and Koblenz (LMWL_{Koblenz}, blue line). The black arrows indicate natural processes of evapotranspirative enrichment of leaf water along local evaporation lines (LELs), biosynthetic fractionation during biomarker synthesis and the temperature effect on the source water isotope composition (\sim precipitation). Grey lines indicate the parallel distance between the individual reconstructed evaporative site leaf water points and the LMWL_{Germany}, expressed as $d = \delta^2\text{H} - 7.72 \cdot \delta^{18}\text{O}$. The difference between the d excesses of the leaf water and source water can serve as proxy for mean daytime vegetation period relative humidity (RH_{dv} ; red double arrow).

proxies seems to be challenging. Similarly, $\delta^{15}\text{N}$ has been investigated as proxy for sedimentary organic matter origin (Meyers and Ishiwatari, 1993; Meyers and Lallier-Vergés, 1999; Wolfe et al., 1999). However, numerous processes that may have an influence, like nitrogen uptake by plants, various nitrogen sources, discrimination during denitrification and diagenesis, complicate the use of $\delta^{15}\text{N}$ as a direct source determination proxy.

Despite the uncertainties presented above, concerning the origin of bulk sedimentary organic matter in the Gemündener Maar, the origin of the sedimentary biomarkers, namely n -alkanes and sugars, needs to be addressed. This is crucial because aquatic biomarkers incorporate the isotope composition of lake water, whereas terrestrial biomarkers incorporate the isotope composition of leaf water (Huang et al., 2004; Kahmen et al., 2013; Mügler et al., 2008; Sachse et al., 2004, 2012; Sauer et al., 2001; Tuthorn et al., 2014; Zech et al.,

2013b, 2014b). With regard to the n -alkane biomarkers, high amounts of the chain lengths $n\text{-C}_{27}$ and $n\text{-C}_{29}$ are characteristic of the Gemündener Maar sediments. Such patterns are typical of epicuticular leaf wax layers of higher terrestrial plants (e.g. Eglinton and Hamilton, 1967). With regard to the sugar biomarkers, they were previously studied in detail by Hepp et al. (2016). According to the authors' own results and a compilation from the literature (including, e.g., Jia et al., 2008; Prietzel et al., 2013; Zech et al., 2012, 2014b), relatively high amounts of arabinose are a good indicator of a primarily terrestrial origin (higher vascular plants) of the sugars. This interpretation is in agreement with the Gemündener Maar being a small lake with densely forested steep crater walls (Fig. 1c). We therefore conclude and suggest that arabinose as well as $n\text{-C}_{27}$ and $n\text{-C}_{29}$ in our Gemündener Maar record are primarily of terrestrial rather than

aquatic origin and thus reflect $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ rather than $\delta^2\text{H}/\delta^{18}\text{O}_{\text{lake-water}}$.

3.2 Reconstructing leaf water isotope composition based on $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$

The $\delta^2\text{H}$ depth profiles reveal variations of -222‰ to -134‰ and -220‰ to -147‰ for $n\text{-C}_{27}$ and $n\text{-C}_{29}$, respectively (Fig. 4a). Their $\delta^2\text{H}$ patterns correlate highly significantly with each other ($r = 0.7$, $p < 0.001$, $n = 59$). Weighted mean $\delta^2\text{H}$ values were calculated using the relative amounts of $n\text{-C}_{27}$ and $n\text{-C}_{29}$. The Younger Dryas is characterised by the most negative $\delta^2\text{H}$ values (mean of -193‰), whereas the Allerød, the Preboreal and the Boreal yielded less negative values (-182‰ , -178‰ and -171‰ , respectively). Still, the Holocene part also reveals two pronounced $\delta^2\text{H}$ minima. Overall, our Gemündener Maar $\delta^2\text{H}_{n\text{-alkane}}$ resembles very well the $\delta^2\text{H}$ $n\text{-C}_{29}$ record of Rach et al. (2014) for Meerfelder Maar close by.

The $\delta^{18}\text{O}$ values for arabinose, xylose and fucose range from 28‰ to 41‰ , 26‰ to 45‰ and 27‰ to 46‰ , respectively (Fig. 4b). They reveal similar trends overall (arabinose vs. xylose: $r = 0.7$, $p < 0.001$, $n = 59$; arabinose vs. fucose: $r = 0.8$, $p < 0.001$, $n = 59$; xylose vs. fucose: $r = 0.8$, $p < 0.001$, $n = 59$). All sugar records show a clear shift to more positive values at the Younger Dryas–Holocene transition. While xylose and fucose exhibit a change of $\sim 8\text{‰}$ and 7‰ , arabinose $\delta^{18}\text{O}$ values show a less pronounced shift of $\sim 3\text{‰}$ (changes are based on the mean $\delta^{18}\text{O}$ values for the Younger Dryas compared to the Preboreal/Boreal period). Xylose is however slightly more negative throughout the Allerød and Younger Dryas compared to arabinose and fucose. Consistently less pronounced changes can be observed for the Allerød–Younger Dryas transition of 1.9‰ , 1.7‰ and 0.9‰ for xylose, fucose and arabinose, respectively (based on the mean $\delta^{18}\text{O}$ values for the Allerød compared to the Younger Dryas). A distinct minimum during the early Preboreal (633 cm depth) characterises all three $\delta^{18}\text{O}$ sugar records.

The isotope compositions of leaf wax $n\text{-alkanes}$ and leaf (hemi-)celluloses from higher plants are known to be strongly related to the water in which they are biosynthesised. They basically reflect the isotope composition of leaf water during photosynthetic activity (Barbour and Farquhar, 2000; Cernusak et al., 2005; Kahmen et al., 2013; Sachse et al., 2012). Hence, the isotope signature of the paleo leaf water, $\delta^{18}\text{O}_\text{l}$ and $\delta^2\text{H}_\text{l}$, respectively, can be reconstructed by using biosynthetic fractionation factors (Fig. 5; Eqs. 8 and 9). For this purpose, fractionation factors of -160‰ for the $n\text{-alkanes}$ $n\text{-C}_{27}$ and $n\text{-C}_{29}$ ($\varepsilon_{\text{bio}}^2$; Sachse et al., 2012; Sessions et al., 1999) and $+27\text{‰}$ for the hemicellulose sugar arabinose ($\varepsilon_{\text{bio}}^{18}$; Cernusak et al., 2003; Schmidt et al., 2001; Sternberg et al., 1986; Yakir and DeNiro, 1990) seem to be appropriate (Eqs. 8 and 9).

$$\delta^{18}\text{O}_\text{l} = (\delta^{18}\text{O}_{\text{arabinose}} - \varepsilon_{\text{bio}}^{18}) / (1 + \varepsilon_{\text{bio}}^{18}/1000) \quad (8)$$

$$\delta^2\text{H}_\text{l} = (\delta^2\text{H}_{n\text{-alkane}} - \varepsilon_{\text{bio}}^2) / (1 + \varepsilon_{\text{bio}}^2/1000) \quad (9)$$

From the study of tree rings, it is known that stem cellulose does not show the full leaf water ^{18}O enrichment signal. Barbour and Farquhar (2000) related this signal dampening to the proportion of unenriched source water contributing to the local synthesis water (p_x) and to the proportion of exchangeable oxygen during cellulose synthesis (p_{ex}). The latter is often assumed to be rather constant around 0.40, as estimated from leaf and wood cellulose of *Eucalyptus globulus* and values compiled from the literature (Cernusak et al., 2005), meaning that around 40 % of the oxygens in the stem cellulose exchanged. Already Helliker and Ehleringer (2002) compared the signal transfer from leaf water to the cellulose of tree stems with the signal transfer occurring in grasses. And Liu et al. (2016) reported signal dampening in the range between 34 % and 53 % for the C_4 grass *Cleistogenes squarrosa*.

Figure 4c illustrates that *Poaceae* pollen concentrations ranged between 11 % and 33 % during the Allerød and the Younger Dryas in the Gemündener Maar record. Hence, a correction for the ^{18}O signal dampening may be required in order to take these vegetation changes into consideration. A respective correction procedure based on mass balance considerations is given in Eq. (10) in order to adjust $\delta^{18}\text{O}_\text{l}$ to $\delta^{18}\text{O}_\text{l}^\#$:

$$\delta^{18}\text{O}_\text{l}^\# = \left\{ \left(\delta^{18}\text{O}_\text{l} - \delta^{18}\text{O}_\text{s} \right) / \left[f_{\text{non-grasses}} + (1 - 0.4) - f_{\text{non-grasses}} \cdot (1 - 0.4) \right] \right\} + \delta^{18}\text{O}_\text{s}. \quad (10)$$

The correction presented in Eq. (10) is based on assumptions that 40 % (0.4) of the leaf water enrichment is lost during hemicellulose biosynthesis of grass leaves, which is well within the range of values presented in the literature for cellulose synthesis in tree rings and grasses (Cernusak et al., 2005; Liu et al., 2016). Furthermore, the *Poaceae* pollen concentration in percentage is used to calculate the fraction of non-grassy pollen ($f_{\text{non-grasses}} = (100 - \text{Poaceae})/100$) corresponding to the non-grassy biomarker contribution, which may serve as a rough approximation. For a paleo application, $\delta^{18}\text{O}_\text{s}$ remains a priori unknown. Therefore, the intercept between the individual LELs (Eq. 6) and the LMWL of Germany were used to generate $\delta^{18}\text{O}_\text{s}$ values. Note that the signal dampening effect described here for cellulose synthesis is likely not fully applicable to our approach using the sugar biomarker arabinose. In fact, pentoses like arabinose are biosynthesised via decarboxylation of the carbon at position six (C6) from glucose (Altermatt and Neish, 1956; Burget et al., 2003; Harper and Bar-Peled, 2002). Waterhouse et al. (2013) showed that the oxygens at C6 position in glucose moieties are most strongly affected by the exchange with a local water medium of 80 %, as indicated by

heterotrophic cellulose synthesis. Thus, arabinose has lost a strongly exchanged (dampened) oxygen and the remaining pentose shows less ^{18}O signal dampening.

With regard to the $\varepsilon_{\text{bio}}^2$ value of -160‰ , this biosynthetic fractionation factor is confirmed by climate chamber studies of dicotyledonous plants (Kahmen et al., 2011, 2013; Tipple et al., 2015). However, the latter studies also reveal a range of $\sim 35\text{‰}$, interpreted as species-specific effects during n -alkane biosynthesis. The difference between dicotyledonous and monocotyledonous C_3 plants is much more pronounced the regarding the degree to which the leaf water isotope enrichment is transferred into leaf n -alkanes (Gamarra et al., 2016; Kahmen et al., 2013). While dicotyledonous plants show signal transfer rates of 96 % on average (Kahmen et al., 2013), a larger range of between 38 % and 61 % is found for monocotyledonous plants (Gamarra et al., 2016). The latter implies that 39 % to 62 % of the ^2H leaf water enrichment is not recorded by the n -alkanes of grasses. Hence, like for $\delta^{18}\text{O}$, a correction may be requested to account for grass-derived n -alkanes:

$$\delta^2\text{H}_l^{\#} = \left\{ \left(\delta^2\text{H}_l - \delta^2\text{H}_s \right) / \left[f_{\text{non-grasses}} + (1 - 0.5) \right] - f_{\text{non-grasses}} \cdot (1 - 0.5) \right\} + \delta^2\text{H}_s, \quad (11)$$

where $\delta^2\text{H}_l^{\#}$ are the grass-corrected $\delta^2\text{H}_l$ values. The $\delta^2\text{H}_s$ values and the non-grassy pollen fraction are defined as in Eq. (10). The mass balance correction presented in Eq. (11) is based on assumptions that only 50 % of the leaf water enrichment is incorporated by the n -alkanes during biosynthesis in grass leaves.

In summary, the discussion outlined above allows reconstructing $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ (and thus RH results with Eq. 5) for four scenarios (see also Table 1): (i) without signal dampening, (ii) with grass-corrected $\delta^2\text{H}$ values, (iii) with grass-corrected $\delta^{18}\text{O}$ values, and (iv) with grass-corrected $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values.

3.3 Reconstructing relative humidity based on the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach

The biomarker-based leaf water values ($\delta_l = \delta^2\text{H}_l$, $\delta^{18}\text{O}_l$ via Eqs. 8 and 9) result in d excess values of leaf water (d_l) ranging between -125‰ and -30‰ (Figs. 5 and 6a). This is well within the range that can be expected. For instance, Voelker et al. (2014) reported “deuterium deviations” (calculated as d excess of leaf water minus 10‰) ranging from 0‰ to -200‰ . And Mayr (2002) conducted climate chamber experiments with *Vicia*, *Brassica* and *Eucalyptus* during his dissertation and measured $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of leaf water ($\delta^{18}\text{O}_{\text{leaf-water}}$ and $\delta^{18}\text{O}_{\text{sugars}}$ are published in Zech et al., 2014b). Accordingly, d excess of leaf water ranged from -38‰ to -171‰ and correlates highly significantly with RH (ranging from 21 % to 68 %).

Using the Gemündener Maar d_l values as input for Eq. (5), RH values during daytime and vegetation period (RH_{dv}) can be calculated (scenario 1 in Table 1). Reconstructed RH_{dv} values range from 32 % to 82 % (Fig. 6b). The error bars covering the Gemündener Maar RH_{dv} record, calculated using pooled d_e standard errors ranging from 3.2‰ to 44.4‰ according Eq. (7), result in an RH uncertainty range of 1.7 % to 23.4 %. The RH_{dv} record shows quite large variability with no clear trend during the Allerød and the first half of the Younger Dryas. The late Younger Dryas and the early and the middle Preboreal are characterised by lower RH values. By contrast, the middle Preboreal reveals the most pronounced RH maximum. The mean reconstructed RH_{dv} value is 53 % (mean RH_{dv} upper limit = 45 %; mean RH_{dv} lower limit = 62 %; see Sect. 2.5). For comparison, the modern RH_{dv} value (06:00 to 19:00 CET from April to October) from the adjacent meteorological station Nürburg-Barweiler (approx. 25 km northeast of Gemündener Maar (GM); hourly data from 1995 to 2015 from Deutscher Wetterdienst, 2016) is 67 % (Fig. 6b). In addition, the range of the reconstructed RH_{dv} values of 50 % agrees well with the modern RH_{dv} variability of 45 %, within a range of 48 % to 93 % (definition and meteorological station details as above). As proposed, in the previous chapter, three correction scenarios can be applied when reconstructing d_l and RH_{dv} values in order to account for ^2H and ^{18}O signal dampening occurring in grasses.

Accordingly, the full correction for grass-derived alkane and sugar biomarkers (scenario 4 in Table 1) results in 0.0 % to 6.3 % (mean 1.8 %) lower RH_{dv} values ($\text{RH}_{\text{dv}}^{\#}$ in Fig. 6b). This corresponds to d_l decreases of 0.0 % to -12.0‰ ($d_l^{\#}$ in Fig. 6a). Such small changes are still far below the pooled analytical standard errors. When only correcting for the ^{18}O signal dampening (scenario 3 in Table 1), d_l values decrease by 0.0 % to -22.7‰ , corresponding to RH decreases of 0.0 % to -12.0‰ ($d_l^{\#}$ and $\text{RH}_{\text{dv}}^{\#}$ in Fig. 6a and b, respectively). By contrast, when only correcting for the ^2H signal dampening (scenario 2 in Table 1), this leads to 0.0 % to 10.6 % more positive and 0.0 % to 5.6 % higher RH_{dv} values (d_l^* and RH_{dv}^* in Fig. 6a and b). Overall, these results suggest that the reconstructed RH_{dv} values are not strongly affected by ^2H and ^{18}O signal dampening of grasses.

We are aware that microclimatic conditions with higher RH values often develop in lower canopy levels of forests (Graham et al., 2014; Parker, 1995). This may result in RH overestimations when applying the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach. However, most leaf biomass is produced at higher canopy levels, which are exposed to sunlight and free-air RH values. This is in agreement with a study of Zech et al. (2015), who investigated n -alkanes in soils of the tropical montane rainforest of Mt Kilimanjaro. There, n -alkanes reflect $\delta^2\text{H}_{\text{leaf-water}}$ as calculated from free-air RH rather than as calculated from nearly saturated ground-level RH.

A basic assumption of our coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach is isotopic equilibrium between

Table 1. Scenarios 1–4 used for reconstructing deuterium (d) excess of leaf water and corresponding RH_{dv} values in order to assess/estimate the effect of variable grass contributions on the reconstructed Gemündener Maar RH record (see also Fig. 6).

Scenario	Leaf water reconstructed from n -alkane or sugar biomarkers	Equations used for leaf water reconstruction	Resulting d excess of leaf water as input for Eq. (5)	Relative air humidity during daytime and vegetation period according Eq. (5)
1	$\delta^2H_1/\delta^{18}O_1$	(8) and (9)	d_1	RH_{dv}
2	$\delta^2H_1^*/\delta^{18}O_1$	(8) and (9) + (11)	d_1^*	RH_{dv}^*
3	$\delta^2H_1/\delta^{18}O_1^\#$	(8) + (10) and (9)	$d_1^\#$	$RH_{dv}^\#$
4	$\delta^2H_1^*/\delta^{18}O_1^\#$	(8) + (10) and (9) + (11)	$d_1^{\#*}$	$RH_{dv}^{\#*}$

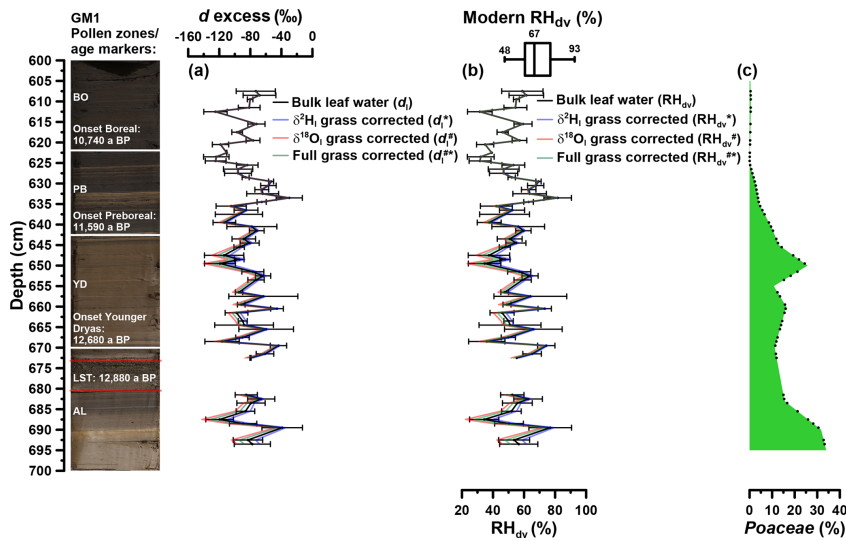


Figure 6. (a) Deuterium (d) excess depth profiles of reconstructed leaf water: d_1 (black line): no correction for grasses; d_1^* (light blue line): δ^2H corrected for grasses; $d_1^\#$ (light red line): $\delta^{18}O$ corrected for grasses; $d_1^{\#*}$ (light green line): δ^2H and $\delta^{18}O$ corrected for grasses. The error bars of d_1 values are calculated according to Eq. (7). (b) Reconstructed RH_{dv} records. Modern RH variability during daytime and vegetation period (RH_{dv}) is displayed as a box plot derived from the adjacent meteorological station Nürburg-Barweiler, using monthly means from April to October between 06:00 and 19:00 CET (based on hourly data from 1995 to 2015; Deutscher Wetterdienst, 2016). The numbers within the box plot represent the maximum, median and minimum values. (c) Depth profile of *Poaceae* pollen. Additionally, the resampled data points (black points) are displayed. The GM1 core picture with the used age markers are displayed on the left. AL: Allerød; LST: Laacher See Tephra; YD: Younger Dryas; PB: Preboreal; BO: Boreal.

plant source water and water vapour. In order to test the robustness of this assumption and respective effects on reconstructed RH values, we used data of Jacob and Sonntag (1991), who measured the isotope composition of precipitation and of atmospheric water vapour in Heidelberg, Germany, during the period 1981 to 1989. The mean difference between the annual weighted means of precipitation (\approx plant source water) and the water vapour averaged over the vegetation period (April–October) was therefore calculated. Such derived apparent fractionation (ϵ_{ap}) amounts to 18.3‰ and 1.57‰ on average for 2H and ^{18}O , respectively. We used this ϵ_{ap} in Eq. (1) instead of the difference $\delta_a - \delta_s$

and recalculated the RH values. This recalculation leads to an average RH change of only -1.7% (± 0.9), which is far below the analytical errors of the d excess of leaf water.

Finally, the stability of the d excess and slope of the LMWL_{Germany} through the past needs to be discussed. According to Stumpp et al. (2014), the long-term d excess of precipitation from 28 sites in Germany does not show pronounced relationships to local climate conditions of the site. All reported values are close to 10‰, which indicates that Atlantic air masses are the main moisture source for Germany (e.g. Rozanski et al., 1993). In addition, the d excess of precipitation from the stations Trier and Koblenz,

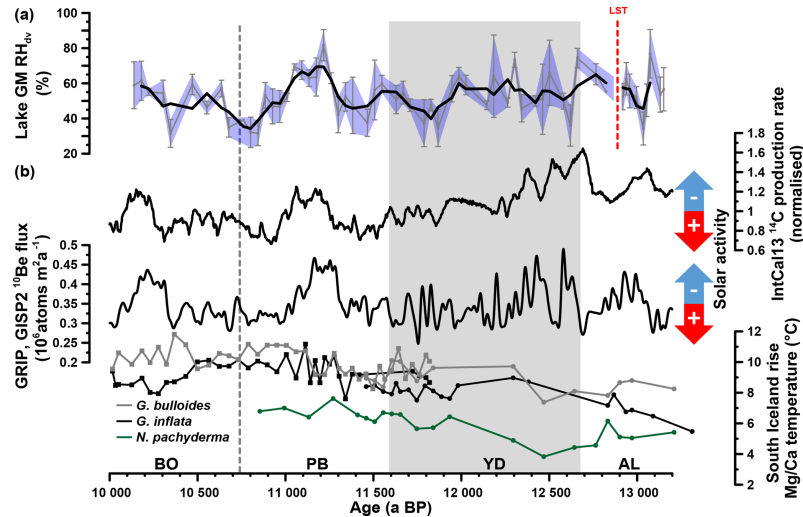


Figure 7. (a) Reconstructed Gemündener Maar (GM) RH_{dv} record. The bold line shows the three-point moving average. Error bars and the blue-shaded area indicate analytical uncertainties calculated according to error propagation (Eq. 7). (b) IntCal13 ^{14}C production rate, Greenland ice-core (GRIP, GISP2) ^{10}Be flux record (both from Muscheler et al., 2014) and South Iceland Rise planktic Mg/Ca-derived water temperatures from RAPiD-12-1K (squares; 10 000 to 11 800 a BP) and RAPiD-15-4P (circles; 10 900 to 13 200 a BP). RAPiD-12-1K and RAPiD-15-4P *G. bulloides* and *G. inflata* data from Thoralley et al. (2009) and Thoralley et al. (2010), respectively. RAPiD-15-4P *N. pachyderma* data from Thoralley et al. (2011). Note that each record is plotted on its own timescale (for planktonic Mg/Ca data, see Thoralley et al., 2009, 2010; for ^{10}Be data on GICC05, see Rasmussen et al., 2006; for ^{14}C data on IntCal13 calibration curve, see Reimer et al., 2013; for RH_{dv} data on GM age–depth model, see Fig. 2d). AL: Allerød, LST: Laacher See Tephra, YD: Younger Dryas, PB: Preboreal and BO: Boreal.

which are close to the Gemündener Maar, reveal rather small variability on a monthly, annual and long-term basis. For Trier monthly averaged d excess values (March to October) range from 5.3‰ to 8.7‰. Annually weighted mean d excess values range from 1.9‰ to 10.6‰, and the long-term weighted mean is 6.7‰ (± 2.2); for Koblenz the d excess values range between 2.1‰ and 6.4‰ and 1.4‰ and 8.7‰, and the long-term weighted mean is 4.1‰ (± 1.8) (derived from IAEA/WMO, 2018). Finally, d excess variability in Greenland and Antarctic ice cores does not exceed 4‰ over the timescale relevant here (Masson-Delmotte et al., 2005; Stenni et al., 2010). In addition, paleowater samples from Europe suggest that the d excess of precipitation was rather constant throughout the past 35 000 years, which implies that the principle atmospheric circulation patterns over the European continent did not change substantially (Rozanski, 1985). In summary, the variations in the slope of the LMWL of Germany are assumed to be rather small over longer timescales.

The detailed discussions in the above three sections address numerous uncertainties when using the coupled $\delta^2H_n\text{-alkane}-\delta^{18}O_{\text{sugar}}$ paleohygrometer approach. Conclusively, the reconstructed RH_{dv} history of the Gemündener Maar seems, however, robust enough to infer reliable paleoclimatic or hydrologic conclusions.

3.4 How dry was the Younger Dryas in western Europe?

While it is well known that the Younger Dryas was a cold spell occurring in the Northern Hemisphere during the Late Glacial (Denton et al., 2010; Heiri et al., 2014; Isarin and Bohncke, 1999), there is much less clear evidence concerning moisture supply or availability and RH changes during the Younger Dryas. The Gemündener Maar RH_{dv} record suggests quite some variability but on average moderate RH_{dv} conditions of $\sim 56\%$ during the end of the Allerød and the first half of the Younger Dryas. This is within the range of modern RH_{dv} values (Fig. 6b). In the second half of the Younger Dryas, a clear RH_{dv} decrease of $\sim 11\%$ occurred (Fig. 7a). Such a two phasing of the Younger Dryas has been suggested previously based on multiproxy climate data for western Europe (Isarin et al., 1998). In more detail, Isarin et al. (1998) reported a cold and humid first phase being followed by drier and warmer conditions. It is moreover speculated that a shift in the mean sea-ice margin during winter in the North Atlantic Ocean slightly to the north could have caused this two phasing. Reduced cyclonic activity and precipitation thereby primarily affected western Europe because this region was situated at the southern margin of the main storm tracks during the first Younger Dryas period (Isarin et al., 1998). The authors also presented evi-

dence for the strengthening of the westerly winds in western Europe as consequence of northward-shifted North Atlantic Ocean sea-ice margin during the late Younger Dryas period. This contradicts, however, with the interpretation of the Meerfelder Maar sedimentary record. Here, the thicker varves during the early Younger Dryas (between 12 680 and 12 240 varve aBP) are used along with geochemical results as indicator of stronger winter winds (Brauer et al., 2008). In line with this, Brauer et al. (1999) interpreted high biogenic opal contents and *Pediastrum* remains concentrations during the early Younger Dryas as enhanced aquatic productivity due to an increased nutrient supply caused by soil erosion and the reworking of littoral sediments. The varve formation throughout the second Younger Dryas period (between 12 240 and 11 590 varve aBP) is interpreted to be mainly controlled by snowmelt-driven surface runoff (Brauer et al., 1999). Moreover, the authors speculate if during that time the Meerbach began to drain into the Meerfelder Maar, which could be possibly linked to enhanced precipitation amounts. In summary, the interpretations derived from the Younger Dryas sediments of the Meerfelder Maar by Brauer et al. (2008, 1999) seem neither to be in accordance with the results of Isarin et al. (1998) nor with the established RH_{dv} record of the Gemündener Maar (Fig. 7a).

Recently, Rach et al. (2017) reconstructed RH changes and generally dry Younger Dryas climatic conditions by investigating δ^2H of terrestrially versus aquatically derived n -alkanes (published in Rach et al., 2014) from the Meerfelder Maar archive. At the current state of research, it can only be speculated about the reasons for this discrepancy, with our Gemündener Maar RH record not corroborating an overall dry Younger Dryas. While the uncertainties of the coupled $\delta^2H_{n\text{-alkane}}-\delta^{18}O_{\text{sugar}}$ paleohygrometer approach were discussed in detail in the previous sections, in our opinion the most important uncertainties affecting the dual-biomarker approach of Rach et al. (2014, 2017) are the following. First, lake water is assumed to reflect δ^2H of precipitation. Indeed, Holzmaar, which seems to be comparable to the Meerfelder Maar at least for the drainage conditions via one creek, shows a difference of 7.4‰ in δ^2H between inflow and lake water (Sachse et al., 2004). This lake water enrichment is likely to have been variable in the past, especially when including the speculation concerning the drainage of the Meerbach during the Younger Dryas (Brauer et al., 1999). Second, $n\text{-}C_{23}$ is interpreted to be of aquatic origin (from *Potamogeton*) and used for reconstructing $\delta^2H_{\text{lake-water}}$. However, there is increasing evidence that $n\text{-}C_{23}$ is also of terrestrial origin (Rao et al., 2014). For instance, Aichner et al. (2018) have recently shown for a lake in Poland that $n\text{-}C_{23}$ shows a variable mixture of aquatic and terrestrial origin in those Late Glacial and Early Holocene sediments. And birch as a pioneering and one of the dominant tree species during Late Glacial reforestation of central Europe is known to produce considerable amounts of mid-chain n -alkanes (Tarasov et al., 2013). Although they are not included in the latter publica-

tion, $n\text{-}C_{23}$ concentrations of *Betula exilis* and *Betula pendula* reached 653 and even 2323 $\mu\text{g g}^{-1}$ in that study. This is highly relevant, because the biosynthetic fractionation factor of aquatic n -alkanes is much smaller than the one of terrestrial n -alkanes. Minor changes in the contribution of terrestrial vs. aquatic n -alkanes will thus have a considerable impact on the reconstructed δ^2H $n\text{-}C_{23}$ record and in turn on reconstructed RH values when applying the dual-biomarker approach. Finally, it may worth acknowledging that Sachse et al. (2004) found no significant correlation for δ^2H of $n\text{-}C_{23}$ and lake water and precipitation along a European lake surface transect.

Also recently and also applying the dual-biomarker approach, Muschitiello et al. (2015) studied Younger Dryas lake sediments from Hässeldala Port in southern Sweden. Here, the authors used δ^2H of $n\text{-}C_{21}$ as a proxy for lake water and summer precipitation. The calculated difference between terrestrial and aquatic n -alkane δ^2H values suggests more humid conditions at the beginning of the Younger Dryas followed by a more or less steady trend towards drier conditions, peaking around 11 700 aBP (Muschitiello et al., 2015). Within age uncertainties, this would be in line with the Gemündener Maar RH_{dv} minimum between $\sim 11\,700$ and 11 900 aBP. Last but not least, Gázquez et al. (2018) analysed triple oxygen and hydrogen isotopes of gypsum in the southern Pyrenees and thus reconstructed RH changes. Again, more humid conditions are reported for the beginning of the Younger Dryas.

In search of possible drivers or mechanisms for the observed Gemündener Maar RH_{dv} record, we came across the ^{14}C production and ^{10}Be flux rates (Fig. 7b), derived from IntCal13 and the Greenland ice cores (GRIP, GISP2), respectively (Muscheler et al., 2014). These records are commonly interpreted in terms of solar activity (and thus insolation) changes (Stuiver and Braziunas, 1988; Vonmoos et al., 2006) and reveal striking similarities with our Gemündener Maar RH_{dv} record. For instance, all three records reveal quite high centennial-scale variability during the Allerød and the first half of the Younger Dryas. Generally low RH_{dv} values during the second half of the Younger Dryas and the Early Preboreal coincide with high solar activity, whereas the pronounced RH_{dv} maximum from 11 260 to 11 050 aBP coincides within age uncertainties with a pronounced solar activity minimum (Fig. 7). We dub this wet period the “Preboreal Humid Phase”, which should not be confused with the Preboreal Oscillation (Björck et al., 1997). The Preboreal Oscillation is a short cold event recorded in Greenland ice cores $\sim 11\,400$ ka (Rasmussen et al., 2007) and led to more arid conditions at least in the Netherlands according to palynological results (Bos et al., 2007; van der Plicht et al., 2004). These pollen records also show the existence of a pronounced humid phase thereafter, thus corroborating the Preboreal Humid Phase. Widespread glacial advances in the Alps are also attributed to the Preboreal Oscillation (Moran et al., 2017). However, given the dating uncertainties they

may actually rather reflect increased precipitation during the Preboreal Humid Phase.

It should be emphasised, that the described similarities between the Gemündener Maar RH_{dv} record and the solar activity records do not allow an a priori causality interpretation. It is widely accepted that the Younger Dryas and the Preboreal Oscillation are related to freshwater forcing in the North Atlantic (e.g. Fisher et al., 2002; Murton et al., 2010; Muschiello et al., 2015). However, the causes and mechanisms responsible for climate and environmental changes during the rest of the Holocene remain vague, and more research including paleoclimate modelling is clearly needed and encouraged to investigate the possible influence of solar activity (Renssen et al., 2007; Rind, 2002). We propose that both the North Atlantic Ocean temperature and solar activity (the latter triggering solar insolation) were the two main drivers for the RH_{dv} variability in central Europe. A key example might be the Preboreal Humid Phase. It can be expected that the North Atlantic Ocean, the main moisture source for central Europe, already had considerably higher temperatures during the Preboreal Humid Phase compared to the Younger Dryas, as indicated by a consistent $\sim 2^\circ\text{C}$ increase in Mg/Ca temperatures derived from planktonic foraminifera (*Globorotalia inflata*, *Globorotalia bulloides* and *Neoglobobulimina pachyderma*) in a marine sediment core south of Iceland (Fig. 7b, Thornalley et al., 2009, 2010, 2011). This led to an enhanced moisture content of the atmosphere. When these wet air masses were transported onto continental Europe, where low solar insolation inhibited warming up and drying of these air masses, pronounced humid climate conditions were established.

4 Conclusions

Referring to the underlying research questions and based on the presented results and the outlined discussion (including the cited literature), the following conclusions have to be drawn.

The terrestrial vs. aquatic origin of bulk sedimentary organic matter cannot be determined unambiguously for the Gemündener Maar. This is caused by the bulk proxies (TOC/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) not being straightforwardly interpretable. By contrast, the alkane biomarkers with the chain-length $n\text{-C}_{27}$ and $n\text{-C}_{29}$ and the sugar biomarker arabinose can be most likely associated with the epicuticular leaf wax layers and the hemicellulose structures of higher terrestrial plants, respectively. Therefore, they are interpreted as originating primarily from leaf material of the Gemündener Maar catchment.

$\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ could be reconstructed from $\delta^2\text{H}_{n\text{-alkane}}$ ($n\text{-C}_{27}$ and $n\text{-C}_{29}$) and $\delta^{18}\text{O}_{\text{sugar}}$ (arabinose) by applying biosynthetic fractionation factors. We acknowledge that the assumption of constant fractionation factors introduces uncertainty as highlighted by the broad literature

discussion. A correction for the signal dampening of leaf water $^2\text{H}/^{18}\text{O}$ enrichment occurring in grasses is possible but seems negligible in the case of the Gemündener Maar record.

The detailed discussion of possible uncertainties of the applied coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ paleohygrometer approach suggests that robust RH reconstructions are possible for the Gemündener Maar record. The reconstructed RH values refer to daytime and vegetation period (RH_{dv}).

The established Gemündener Maar RH_{dv} record supports a two phasing of the Younger Dryas with moderate wet conditions at Allerød level during the first half and drier conditions during the second half of the Younger Dryas. Overall, dry climatic conditions characterising the Younger Dryas could not be corroborated. Unexpectedly, the amplitude of RH_{dv} changes during the Early Holocene was more pronounced than during the Younger Dryas and includes a pronounced Preboreal Humid Phase occurring from $\sim 11\,260$ to $11\,050$ a BP. We propose North Atlantic Ocean temperature and solar activity (and thus insolation) as the main drivers for Late Glacial–Early Holocene RH changes in central Europe and encourage respective paleoclimate modelling studies in order to validate or falsify our proposition.

Data availability. The data are available in the Supplement, including sampling depth, calculated ages, measured $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ values, reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$, d excess of leaf water, RH_{dv} , and $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ values.

Supplement. The supplement related to this article is available online at: <https://doi.org/10.5194/cp-15-713-2019-supplement>.

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Supplement of

How dry was the Younger Dryas? Evidence from a coupled $\delta^2\text{H}$ – $\delta^{18}\text{O}$ biomarker paleohygrometer applied to the Gemündener Maar sediments, Western Eifel, Germany

Johannes Hepp et al.

Correspondence to: Johannes Hepp (johannes-hepp@gmx.de)

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Tab. S1: Weighted mean $\delta^2\text{H}$ values of leaf wax-derived n -alkanes ($n\text{-C}_{27}$ and $n\text{-C}_{29}$) and $\delta^{18}\text{O}$ values of hemicellulose-derived sugar (arabinose). The reported standard errors represent the analytical uncertainties. Also calculated/reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$, d-excess of leaf water, mean daytime vegetation period relative humidities (RH_{dv}), and $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source-water}}$ values are displayed. The reported uncertainties of d-excess and RH represent expanded uncertainties calculated using the uncertainty propagation law.

Depth [cm]	Age [a BP]	Measured		Calculated/Reconstructed					
		$\delta^2\text{H}_{n\text{-alkane}}$ [‰]	$\delta^{18}\text{O}_{\text{sugar}}$ [‰]	$\delta^2\text{H}_{\text{leaf-water}}$ [‰]	$\delta^{18}\text{O}_{\text{leaf-water}}$ [‰]	d-excess of leaf water [‰]	RH_{dv} [%]	$\delta^2\text{H}_{\text{source-water}}$ [‰]	$\delta^{18}\text{O}_{\text{source-water}}$ [‰]
607.5	10139	-163.7 ± 3.3	36.1 ± 0.9	-4.4	8.9	-73 ± 25	59 ± 13	-49	-7
608.5	10180	-156.8 ± 2.7	36.6 ± 1.2	3.9	9.3	-68 ± 21	61 ± 11	-38	-6
609.5	10222	-159.0 ± 0.5	37.8 ± 0.7	1.2	10.5	-80 ± 4	55 ± 2	-48	-7
611.5	10305	-205.3 ± 1.8	30.7 ± 0.5	-53.9	3.6	-81 ± 14	54 ± 7	-104	-14
612.5	10346	-205.4 ± 1.9	36.4 ± 0.1	-54.1	9.2	-125 ± 15	32 ± 8	-129	-17
615.5	10470	-146.0 ± 1.4	38.8 ± 0.3	16.6	11.5	-72 ± 11	59 ± 6	-28	-4
617.5	10553	-170.9 ± 0.9	38.2 ± 0.9	-13.0	10.9	-97 ± 7	46 ± 4	-72	-10
619.5	10636	-159.2 ± 1.2	37.3 ± 0.2	0.9	10.0	-77 ± 9	57 ± 5	-46	-7
620.5	10678	-173.7 ± 1.4	40.6 ± 0.5	-16.3	13.2	-118 ± 11	35 ± 6	-87	-12
622.5	10761	-200.1 ± 0.0	35.3 ± 0.2	-47.7	8.1	-110 ± 0	39 ± 0	-114	-15
623.5	10802	-194.9 ± 2.1	37.9 ± 1.0	-41.5	10.7	-124 ± 16	32 ± 9	-115	-16
624.5	10844	-202.8 ± 1.7	36.8 ± 0.4	-50.9	9.5	-125 ± 13	32 ± 7	-125	-17
625.5	10885	-166.8 ± 1.8	37.1 ± 0.4	-8.0	9.8	-84 ± 14	53 ± 7	-59	-8
626.5	10927	-174.6 ± 2.4	37.4 ± 0.5	-17.4	10.1	-96 ± 19	47 ± 10	-75	-10
627.5	10968	-179.2 ± 2.2	36.7 ± 1.0	-22.9	9.5	-96 ± 17	47 ± 9	-81	-11
628.5	11010	-171.6 ± 0.6	36.6 ± 1.0	-13.8	9.4	-86 ± 5	52 ± 2	-66	-9
629.5	11051	-170.4 ± 0.2	32.4 ± 2.7	-12.4	5.3	-53 ± 3	69 ± 2	-46	-7
630.5	11092	-173.0 ± 1.3	32.5 ± 0.6	-15.4	5.3	-57 ± 10	68 ± 5	-51	-7
631.5	11134	-170.2 ± 1.1	34.1 ± 0.4	-12.1	6.9	-65 ± 9	63 ± 5	-52	-7
632.5	11175	-169.0 ± 2.7	34.1 ± 0.2	-10.7	6.9	-64 ± 21	64 ± 11	-50	-7
633.5	11217	-174.8 ± 2.2	28.6 ± 0.6	-17.7	1.6	-30 ± 17	82 ± 9	-38	-6
634.5	11258	-169.2 ± 0.8	34.2 ± 0.6	-11.0	7.0	-65 ± 6	63 ± 3	-51	-7
635.5	11300	-189.2 ± 2.5	36.3 ± 1.6	-34.8	9.1	-105 ± 19	42 ± 10	-98	-13
636.5	11341	-177.5 ± 2.0	35.6 ± 0.4	-20.9	8.4	-86 ± 15	52 ± 8	-73	-10
637.5	11383	-170.1 ± 3.9	38.0 ± 1.5	-12.0	10.7	-95 ± 30	47 ± 16	-69	-10
639.5	11466	-172.5 ± 1.9	40.1 ± 1.4	-14.8	12.8	-113 ± 15	38 ± 8	-83	-11
640.5	11507	-165.1 ± 4.2	36.6 ± 0.3	-6.0	9.3	-78 ± 32	56 ± 17	-54	-8
641.5	11549	-181.7 ± 1.2	33.1 ± 0.7	-25.8	5.9	-72 ± 9	60 ± 5	-70	-10
643.5	11630	-202.3 ± 2.0	32.1 ± 0.2	-50.4	4.9	-88 ± 15	51 ± 8	-104	-14
644.5	11669	-181.1 ± 1.6	34.4 ± 0.3	-25.1	7.2	-81 ± 12	55 ± 6	-74	-10
645.5	11709	-199.1 ± 0.9	33.4 ± 0.4	-46.6	6.2	-94 ± 7	48 ± 4	-104	-14
647.5	11788	-200.4 ± 3.3	35.7 ± 0.5	-48.1	8.5	-114 ± 25	37 ± 13	-116	-16
648.5	11828	-193.5 ± 0.8	34.2 ± 0.2	-39.9	7.0	-94 ± 6	48 ± 3	-97	-13
649.5	11867	-212.3 ± 2.6	34.6 ± 0.3	-62.3	7.4	-119 ± 20	35 ± 10	-134	-18
651.5	11947	-186.7 ± 1.8	33.0 ± 0.2	-31.8	5.8	-77 ± 14	57 ± 7	-79	-11
652.5	11986	-188.8 ± 1.3	30.9 ± 0.4	-34.3	3.8	-64 ± 10	64 ± 5	-74	-10
653.5	12026	-186.3 ± 1.4	32.5 ± 0.5	-31.3	5.4	-73 ± 11	59 ± 6	-76	-10
656.5	12145	-196.4 ± 0.6	33.8 ± 0.7	-43.4	6.6	-95 ± 5	48 ± 2	-101	-14
657.5	12185	-167.2 ± 5.8	34.3 ± 0.4	-8.6	7.1	-63 ± 44	64 ± 23	-48	-7
659.5	12264	-201.2 ± 0.6	32.7 ± 0.7	-49.0	5.5	-92 ± 5	49 ± 3	-105	-14
660.5	12303	-191.6 ± 1.1	28.1 ± 0.8	-37.6	1.0	-46 ± 8	73 ± 4	-67	-9
661.5	12343	-210.7 ± 1.9	32.0 ± 0.7	-60.4	4.8	-98 ± 15	46 ± 8	-119	-16
663.5	12422	-197.5 ± 0.7	33.0 ± 0.5	-44.6	5.9	-90 ± 6	50 ± 3	-99	-13
664.5	12462	-189.2 ± 4.9	34.1 ± 0.7	-34.8	6.9	-88 ± 38	51 ± 20	-88	-12
665.5	12502	-171.2 ± 4.6	33.2 ± 0.1	-13.3	6.0	-60 ± 35	66 ± 19	-50	-7
667.5	12581	-185.8 ± 1.1	34.9 ± 0.4	-30.8	7.7	-90 ± 9	50 ± 5	-85	-12
668.5	12621	-216.0 ± 2.9	33.6 ± 0.1	-66.6	6.4	-116 ± 22	36 ± 12	-136	-18
669.5	12660	-173.6 ± 1.4	30.7 ± 0.0	-16.2	3.6	-44 ± 11	74 ± 6	-44	-6
671.5	12766	-172.7 ± 1.5	33.1 ± 0.4	-15.1	6.0	-61 ± 11	65 ± 6	-53	-8
672.5	12823	-192.5 ± 0.0	32.5 ± 0.8	-38.7	5.3	-80 ± 1	55 ± 0	-87	-12
673.5	LST	n.d. ± n.d.	n.d. ± n.d.	n.d.	n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d.	n.d.
679.5	LST	n.d. ± n.d.	n.d. ± n.d.	n.d.	n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d.	n.d.
681.5	12919	-176.8 ± 1.8	35.7 ± 0.4	-20.0	8.5	-85 ± 14	52 ± 8	-72	-10
682.5	12938	-175.0 ± 2.3	33.4 ± 0.4	-17.8	6.2	-66 ± 17	63 ± 9	-59	-8
683.5	12957	-177.0 ± 2.4	34.8 ± 1.1	-20.2	7.6	-79 ± 18	56 ± 10	-68	-9
685.5	12996	-179.6 ± 1.5	35.4 ± 0.1	-23.3	8.2	-86 ± 12	52 ± 6	-76	-10
687.5	13035	-221.3 ± 2.3	33.2 ± 0.3	-73.0	6.0	-120 ± 18	34 ± 9	-144	-19
688.5	13054	-184.3 ± 2.3	35.0 ± 0.5	-28.9	7.8	-89 ± 18	50 ± 9	-83	-11
689.5	13074	-177.1 ± 3.4	29.5 ± 0.6	-20.3	2.4	-39 ± 26	77 ± 14	-46	-7
692.5	13132	-182.3 ± 2.4	34.5 ± 0.6	-26.6	7.3	-83 ± 19	54 ± 10	-77	-11
693.5	13151	-169.0 ± 3.0	35.9 ± 0.1	-10.7	8.6	-77 ± 23	57 ± 12	-58	-8

n.d. = not determined; LST = Laacher See Tephra

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1 **Late Glacial to Early Holocene $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ records from Lake Bergsee, Black Forest,**
2 **Germany – potential and limitations**

3
4 Johannes Hepp^{a,b,1,#}, Lucas Kämpf^{c,2}, Damien Rius^d, Mario Tuthorn^{b,3}, Lucas Bittner^b, Laurent Millet^d, Fanny
5 Duprat-Oualid^d, Bruno Glaser^b, Michael Zech^{b,e,4}

6
7 ^a*Chair of Geomorphology and BayCEER, University of Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth,*
8 *Germany*

9 ^b*Institute of Agronomy and Nutritional Sciences, Soil Biogeochemistry, Martin-Luther-University Halle-*
10 *Wittenberg, Von-Seckendorff-Platz 3, D-06120 Halle, Germany*

11 ^c*Institute for Soil Science and Site Ecology, Site Ecology and Plant Nutrition, Pienner Strasse 19, D-01737*
12 *Tharandt, Germany*

13 ^d*CNRS UMR 6249, Laboratoire Chrono-Environnement, UFR des Sciences et Techniques, Université de*
14 *Bourgogne-Franche-Comte, 16 Route de Gray, F-25030 Besancon, France*

15 ^e*Institute of Geography, Chair of Landscape- and Geoecology, Dresden University of Technology,*
16 *Helmholtzstrasse 10, D-01062 Dresden, Germany*

17
18 [#]*corresponding author: johannes-hepp@gmx.de*

¹ Present Address: *Chair of Geomorphology and BayCEER, University of Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth, Germany*

² Present Address: *CNRS UMR 6249, Laboratoire Chrono-Environnement, UFR des Sciences et Techniques, Université de Bourgogne-Franche-Comte, 16 Route de Gray, F-25030 Besancon, France*

³ Present Address: *Thermo Fisher Scientific, Hanna-Kunath-Str. 11, D-28199 Bremen, Germany*

⁴ Present Address: *Institute of Geography, Chair of Landscape- and Geoecology, Dresden University of Technology, Helmholtzstrasse 10, D-01062 Dresden, Germany*

19 **Keywords**

20 lacustrine sediments, biomarkers, source identification, oxygen isotopes, hydrogen isotopes, leaf water,
21 evaporative enrichment

22

23 **Abstract**

24 During the last decade, compound-specific $\delta^2\text{H}$ analyses of leaf wax-derived *n*-alkane biomarkers became
25 a popular tool in paleoclimate and particularly paleolimnological research. More recently, additionally
26 compound-specific $\delta^{18}\text{O}$ analyses of plant-derived sugar biomarkers emerged as paleoclimate proxy. By
27 applying both tools to the Late Glacial to Early Holocene sedimentary archive of Lake Bergsee, Black Forest,
28 Germany, we aimed at contributing to the paleoclimate reconstruction of Central Europe.

29 A prerequisite for the interpretation of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ records obtained from sedimentary biomarkers is the
30 knowledge about the primarily terrestrial or aquatic origin of the investigated biomarkers. The long-chain
31 *n*-alkanes of Lake Bergsee reflect the vegetation history as derived from pollen results and can be
32 attributed with reasonable certainty to terrestrial sources/plants. Similarly, the high relative abundance
33 of fucose strongly suggests a primarily aquatic origin of the sugar biomarkers. By contrast, the origin of the
34 mid-chain *n*-alkane *n*-C₂₃ is prone to large uncertainty because it can be produced in high amounts by both
35 terrestrial plants such as birch and aquatic organisms. Moreover, a straightforward interpretation of the
36 terrestrial $\delta^2\text{H}_{n\text{-alkane}}$ and the aquatic $\delta^{18}\text{O}_{\text{sugar}}$ records of Lake Bergsee is challenging due to unknown degrees
37 of evapo(transpi)rative enrichment of leaf and lake water, respectively.

38 Finally, we tested the applicability of the recently proposed 'dual-biomarker approach' and the 'coupled
39 $\delta^2\text{H}_{n\text{-alkane}} - \delta^{18}\text{O}_{\text{sugar}}$ approach' as possible tools for reconstructing relative humidity and lake water
40 evaporation. Our discussion concerning possible uncertainties advises, however, caution. In the case of Lake
41 Bergsee, we refrain from applying the 'dual-biomarker approach' because (i) lake water enrichment cannot
42 be excluded but is rather very likely, (ii) *n*-C₂₃ is no robust aquatic biomarker and (iii) the $\varepsilon^2_{\text{bio}}$ value of
43 aquatic *n*-alkanes is an issue of major uncertainty. Minor changes in the contribution of aquatic versus
44 terrestrial *n*-C₂₃ are likely to have a significant influence on the $\delta^2\text{H}$ record of *n*-C₂₃. We also refrain from
45 applying the 'coupled $\delta^2\text{H}_{n\text{-alkane}} - \delta^{18}\text{O}_{\text{sugar}}$ approach', because neither a reliable pure terrestrial nor a
46 reliable pure aquatic $\delta^2\text{H}$ and $\delta^{18}\text{O}$ coupling is possible based on the source identification results for Lake
47 Bergsee.

48

49 1 Introduction

50 The analyses of lipid biomarkers became a popular tool in paleoenvironmental and -climate research (e.g.
 51 Sachse et al. 2012; Diefendorf and Freimuth 2016). In paleolimnological studies, for instance, the hydrogen
 52 isotopic composition from aquatic-derived lipids (aquatic $\delta^2\text{H}$ from short- and mid-chain *n*-alkanes and *n*-
 53 alkanolic acids) from lacustrine sedimentary archives are used for reconstructing the hydrogen isotope
 54 composition of lake water ($\delta^2\text{H}_{\text{lake-water}}$) and consequently precipitation (e.g. Jacob et al. 2007; Seki et al.
 55 2011; Rach et al. 2014; Muschitiello et al. 2015). Due to lake evaporation, lake water can become
 56 isotopically enriched, thus weakening the direct link to the precipitation signal (Hou et al. 2008; Hepp et
 57 al. 2015). Alternatively, leaf wax-derived lipid biomarkers (e.g. long-chain *n*-alkanes and *n*-alkanoic) are
 58 extracted from lacustrine sediments and measured in order to obtain terrestrial compound-specific $\delta^2\text{H}$
 59 values (e.g. Sachse et al. 2006; Rao et al. 2014). Those values in turn reflect the isotope composition of leaf
 60 water from higher terrestrial plants grown in the catchment, which can be related to precipitation
 61 modified by leaf water ^2H enrichment caused by evapotranspiration (Kahmen et al. 2013; Zech et al. 2015).
 62 The extent of leaf and lake water evapo(transpi)rative enrichment is mainly driven by relative humidity
 63 normalized to leaf temperature (as e.g. reviewed by Cernusak et al. 2016) and inflow (input) to evaporation
 64 ratio as well as relative humidity normalized to lake temperature (e.g. review by Gibson et al. 2016). When
 65 leaf/lake water enrichment cannot be ruled out, the interpretation of $\delta^2\text{H}_{n\text{-alkane}}$ -based climate proxies have
 66 to remain often qualitativ. Comparable to the $\delta^2\text{H}_{n\text{-alkane}}$, compound-specific oxygen stable isotope ($\delta^{18}\text{O}$)
 67 analysis of individual sugar biomarkers offer insight into the isotopic composition of precipitation (Zech
 68 and Glaser 2009; Zech et al. 2014b). When the sugars originate from aquatic sources, they reflect lake
 69 water and can thus be interpreted either in terms of reflecting $\delta^{18}\text{O}$ of past precipitation or as
 70 precipitation/evaporation proxy due to lake water evaporative ^{18}O enrichment (Hepp et al. 2015). When
 71 sugars originate primarily from terrestrial sources, they reflect precipitation modified by
 72 evapotranspirative enrichment of leaf water (Tuthorn et al. 2014; Zech et al. 2014a). The recently proposed
 73 coupling of $\delta^2\text{H}_{n\text{-alkane}}$ results with $\delta^{18}\text{O}_{\text{sugar}}$ results (Zech et al. 2013; Tuthorn et al. 2015; Hepp et al. 2017)
 74 as well as the 'dual-biomarker approach' (using the difference between terrestrial and aquatic *n*-alkane
 75 $\delta^2\text{H}$ values; Rach et al. 2017) have the potential to overcome the above mentioned limitation/uncertainty
 76 caused by unknown evapo(transpi)rative enrichment using single $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ records.
 77 Moreover, such coupled approaches allow relative humidity reconstructions and may thus contribute to
 78 respective quantitative paleoclimate research (Eley and Hren 2018; Gázquez et al. 2018).

79 With our study we aimed at contributing to the Late Glacial – Early Holocene paleoclimate reconstruction
 80 of Central Europe by investigating the sedimentary archive of Lake Bergsee in the Black Forest, Germany.

81 More specifically, we present (i) sedimentary bulk (total organic carbon/nitrogen, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and
82 biomarker (*n*-alkanes and sugars) proxies and discuss their potential/limitations for source identification,
83 (ii) $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ records and discuss their potential/limitations for reconstructing paleoclimatic
84 conditions and (iii) discuss the potential/limitations of applying the above introduced 'dual-biomarker
85 approach' and 'coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ approach' to our Lake Bergsee record.

86

87 **2 Material & Methods**

88 **2.1 Lake Bergsee**

89 Lake Bergsee (7°56'11"E, 47°34'20"N) is situated at 382 m a.s.l. on the foothill of the southern Black
90 Forest, Baden Württemberg, Germany (Becker et al. 2006), around 2 km north of Bad Säckingen (Fig. 1A).
91 The modern lake is maximum 335 m long and 250 m wide, representing the small lake surface area with
92 maximum water depth of 13 m. The natural catchment area is rather small with 0.162 km², restricted to
93 the surrounded slopes which are densely covered by forest vegetation. The lake has no natural inflow and is
94 only fed by precipitation and groundwater. The creek Seebächle is the natural outlet of the lake. Since
95 1802/1803 the water level of Lake Bergsee is controlled via a connection to the close-by creek
96 Schöpfenbach, which enlarged the catchment area by 10 km² (Becker et al. 2006). The lake is located in a
97 basin formed by the Riss glacier, embedded in the crystalline basement of mainly pre-Hercynian gneisses
98 (Becker and Angelstein 2004; Becker et al. 2006). Detailed pollen profiles of Lake Bergsee sediments are
99 published by Becker et al. (2006), up to the maximum depth of 20.7 m (covering approximately the last
100 30,000 a). Additionally, the authors show chironomid assemblages and geochemical results. Duprat-Oualid
101 et al. (2017) interpreted pollen results from a master core section between 1571 and 2850 cm
102 (corresponding to an age interval between 14,700 and 45,000 a cal BP) based on a most recent twin coring
103 campaign in November 2013. Pollen analysis, however, was already performed until a master core depth
104 of 1350 cm.

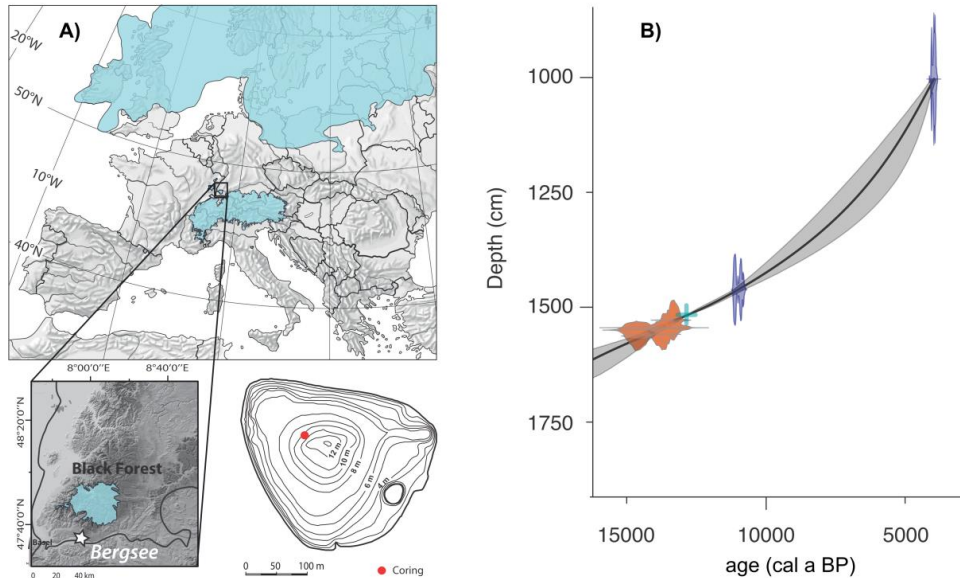


Fig. 1: A) Maps depicting the Lake Bergsee location in Europe and in the Southern Black Forest Region. Blue shaded areas show the glacier extend during the Last Glacial Maximum. Furthermore, the actual bathymetric map of Lake Bergsee is shown, highlighting the coring position with a red dot. B) Lake Bergsee Late Glacial/Holocene age-depth model, comprising the ^{14}C dates obtained by Duprat-Oualid et al. (2017), shown in blue, the Laacher See Tephra is marked with a light blue cross and the orange areas mark 5 newly added ^{14}C ages obtained from microfossils found during the core sampling campaign for this study, see Tab. 1.

Mean annual precipitation over the Lake Bergsee is 1159 mm. Typically, the January is the coldest month within the year, revealing a mean air temperature of 0.9°C. July is in average the warmest month with 19.2°C. Data are means of the measuring period between 1981 to 2010 from the agrometeorological station Bad Säckingen at 339 m a.s.l. (Deutscher Wetterdienst).

2.2 Core details, sampling strategy and age-depth model

In the course of the project “Last Glacial Termination in Europe” two cores were retrieved (BER 13-01 & BER 13-02), close to the Livingston piston core BL2 of Becker et al. (2006), as overlapping twin cores (Duprat-Oualid et al. 2017). Coring was carried out with a Livingston piston corer (UWITEC, Mondsee, Austria) from a floating platform. The master core with 2850 cm length was established using magnetic

123 susceptibility measurements and high-resolution core imaging (Duprat-Oualid et al. 2017). The most
124 recent cored Lake Bergsee sediments covers approximately the last 45,000 a, as derived from the age-
125 depth model (Duprat-Oualid et al. 2017).

126 The samples investigated in this study originate from a 1.50 m long part of BER 13-01 representing a master
127 core section between 1455 and 1605 cm depth. Samples were taken in 1 cm resolution. After sampling,
128 the sediments were dried at 40°C and homogenized (grinded) before further analysis. All laboratory work
129 and measurements were done at the Martin-Luther-University Halle-Wittenberg, Institute of Agronomy
130 and Nutritional Sciences, Group of Soil Biogeochemistry.

131 For detailed information about the age-depth model the reader is referred to Duprat-Oualid et al. (2017).
132 Within this study the age-depth model was refined by ^{14}C analysis of 5 macrofossils found during core
133 sampling (Tab. 1). Radiocarbon analysis and calibration was carried out in the Laboratory for the Analysis
134 of Radiocarbon at the University of Bern, using accelerator mass spectrometry. In summary, the
135 investigated core section is based on 5 ^{14}C macrofossil dates and 2 of wood/needle and wood material in
136 1461.7 and 1563 cm composite depth (Duprat-Oualid et al. 2017), and represents the Late Glacial to Early
137 Holocene transition (16,000 to 10,750 a cal BP; Fig. 1B).

138 **Tab. 1:** New microfossil radiocarbon data obtained from Lake Bergsee sediment core BER 13-01.

composite depth	laboratory reference	uncalibrated ages	calibrated ages
(cm)	(Bern)	(^{14}C a BP)	(cal a BP; 2σ)
1528.2	7064.1.1	$11,489 \pm 178$	$13,384 \pm 343$
1544.7	7065.1.1	$11,651 \pm 407$	$13,782 \pm 1058$
1545.7	7066.1.1	$11,770 \pm 213$	$13,648 \pm 476$
1549.7	7067.1.1	$12,460 \pm 189$	$14,637 \pm 638$
1558.7	7068.1.1	$11,892 \pm 188$	$13,766 \pm 451$

139 Bern = Laboratory for the Analysis of Radiocarbon at the University Bern

140

141 2.3 Bulk sedimentary analysis

142 An EuroVector EA 3000 elemental analyzer (Hekatech, Wegberg, Germany) coupled via a Conflo III
143 Interface to a Delta V Advantage isotope ratio mass spectrometer (IRMS; both from Thermo Fisher
144 Scientific, Bremen, Germany) was used for the analysis of total carbon (TC), total nitrogen (TN), carbon
145 isotope composition ($\delta^{13}\text{C}_{\text{TC}}$) and nitrogen isotope composition ($\delta^{15}\text{N}$). For calibration, standard materials

from the International Atomic Energy Agency (IAEA) and United States Geological Survey (USGS) with known total carbon, nitrogen, ^{13}C and ^{15}N contents were used (IAEA N2, IAEA CH6, IAEA NO3, IAEA CH7, IAEA 305A, USGS 41). The isotope compositions are expressed relative to an international standard in the common δ -notation (e.g. Coplen, 2011; ^{13}C : Vienna Pee Dee Belemnite, VPDB; ^{15}N : atmospheric N_2 , Air).

2.4 Biomarker and compound-specific isotope analysis

Free lipids were extracted 24 h using a soxhlet system (Behr Labor-Technik, R 106 S), constantly rinsed by solvent (dichloromethane:methanol in a ratio of 9:1). After evaporation of the solvent by a rotary evaporator, the total lipid extract was dissolved again and transferred to a pipette column filled with aminopropyl silica gel (Supelco, 45 μm). Three different solvents of increasing polarity (*n*-hexane; dichloromethane:methanol in a ratio of 1:1; diethyl ether + acetic acid in a ratio of 1:19) were used to successively elute the fractions (nonpolar fraction, including *n*-alkanes; more polar fraction, including e.g. alcohols; acids) from the pipette column. Quantification of *n*-alkanes was performed on a GC-2010 series gas chromatograph equipped with a flame ionization detector (GC-FID; Shimadzu, Kyoto, Japan). A C_7 - C_{40} saturated *n*-alkane standard mixture (Supelco 49452-U) in three different concentrations (10, 50 and 100 $\mu\text{g/ml}$) was co-analyzed in each batch several times, and used as quantification standards via linear calibration. The compound-specific $\delta^2\text{H}_{n\text{-alkane}}$ analysis was realized on a Trace GC 2000 coupled to a Delta V Advantage IRMS via a ^2H -pyrolysis reactor (GC IsoLink) and a ConFlo IV interface (all devices from Thermo Fisher Scientific, Bremen, Germany). The reactor temperature was set to 1425°C. Samples were injected with a split/splitless injector, operating in splitless mode. The precision was checked by a standard alkane mixture (*n*- C_{27} , *n*- C_{29} , *n*- C_{33}) with known isotope composition (A. Schimmelmann, University of Indiana), co-injected in three different concentrations after nine sample runs. The samples were analyzed in three- to ninefold repetition and only $\delta^2\text{H}$ results for analytical uncertainty (standard deviation) better than 10‰ are shown, because a higher uncertainty is typically indicative for low concentrations (= measurement areas) and/or not baseline separated peaks. The H_3^+ -correction factor was checked at least before and after a sample batch and stayed stable throughout the measurement period. The stable hydrogen isotope compositions are given in the δ -notation versus Vienna Standard Mean Ocean Water (VSMOW).

The sugar biomarker extraction followed the procedure described by Zech and Glaser (2009). Briefly, from the grinded samples the monosaccharides were released hydrolytically using 4 M trifluoroacetic acid at 105°C for 4 h (Amelung et al., 1996). The solution was cleaned over glass fibre filters, XAD-7 columns and finally over DOWEX 50WX8 columns. After freeze-drying, the samples were split for (i) methyloxime-

trimethylsilyl-derivatisation method (Andrews 1989), which enables the quantification of a large range of sugars, and (ii) methylboronic acid (MBA) derivatization procedure for 1 h at 60°C (Knapp 1979), which ensures that the investigated arabinose (ara), xylose (xyl), fucose (fuc) and rhamnose (rham) yield only one peak in the $\delta^{18}\text{O}$ chromatograms (Gross and Glaser 2004). Quantification of the monosaccharides ara, fuc, galactose (gal), glucose (glu), mannose (man), rham, ribose (rib) and xyl were realized on a GC-FID (Shimadzu, Kyoto, Japan). Compound-specific $\delta^{18}\text{O}_{\text{sugar}}$ measurements were performed with the MBA derivatized samples on a Trace GC 2000 coupled to a Delta V Advantage IRMS via an ^{18}O -pyrolysis reactor (GC IsoLink) and a ConFlo IV interface (all devices from Thermo Fisher Scientific, Bremen, Germany). The samples were measured in threefold repetition. Co-derivatized sugar standard batches are measured in-between, containing ara, fuc, xyl, and rham in various concentrations of known $\delta^{18}\text{O}$ value (Zech and Glaser 2009). The $\delta^{18}\text{O}$ values of the samples were drift- and amount-corrected and a correction for the hydrolytically introduced oxygen atoms on the carbonyl group at the C1 position of the sugar molecules was applied (Zech and Glaser 2009). Standard uncertainties (deviations) for at least triplicate sample measurements (excepted of 11 samples from which only duplicate measurements are available) are on average 1.4, 1.4, 1.6‰ for ara (n = 130), fuc (n = 126) and xyl (n = 124), respectively. Sugars revealing $\delta^{18}\text{O}$ standard deviations higher than 6‰ were omitted from further interpretation, because sugar concentration (= measurement area) was typically too low for robust evaluation (especially for most of the rham peaks), which were finally excluded from further data evaluation. The $\delta^{18}\text{O}$ values of the monosaccharides are expressed in common δ -notation versus the VSMOW.

3 Results & Discussion

3.1 Source identification of bulk sedimentary organic matter

The TC contents of the investigated Lake Bergsee sediment section range between 4 and 28% (Fig. 2A). The TN record highly resembles the TC depth variations ($r^2 = 0.92$, $p < 0.001$, $n = 149$) and is therefore not displayed in Fig. 2. In order to infer information about the source of the sedimentary organic matter, proxies derived from bulk analysis can potentially be used, i.e. TC to TN ratio (TC/TN), $\delta^{13}\text{C}_{\text{TC}}$ and $\delta^{15}\text{N}$ (Fig. 2B to D). The TC/TN ratio range between 7 and 16, with a slightly increasing trend from the top to the bottom of the section (Fig. 2B). Using a threshold of > 12 as indicator for terrestrial input (Prah et al. 1980), no distinctive source can be identified since the bulk sedimentary TC/TN ratios plot all close to 12. Moreover, the TC/TN proxy should not be over-interpreted, because mineralization and degradation are well known to results in very low TC/TN values of terrestrial soils, too (Zech et al. 2007). Lake Bergsee $\delta^{13}\text{C}_{\text{TC}}$ ranges from -30 to -13‰ (Fig. 2C). More positive values than -24‰ in the lower part of the core

cannot be explained with terrestrial C_3 plants as only sedimentary carbon source. Given that a C_4 plant contribution can be excluded (Duprat-Oualid et al. 2017), this points either to allochthonous (aeolian) or autochthonous anorganic carbonate, or to autochthonous organic (aquatic) matter contributing to TC. The $\delta^{15}N$ values range between -1 and 4‰, with a minimum during the Allerød period (Fig. 2D). Albeit this minimum, which covers a composite depth of 1505 to 1535 cm, does not perfectly match the Bølling (BL) and Allerød (AL) TC maximum, it could be interpreted to reflect increased biomass production including N-fixation, be it aquatic or terrestrial. Moreover, numerous further factors such as e.g. denitrification and mineralisation can influence both terrestrial and lacustrine sedimentary $\delta^{15}N$ values (Meyers and Ishiwatari 1993; Zech et al. 2011a). Hence, a robust source identification based on $\delta^{15}N$ values seems to be challenging. Conclusively, a straightforward source identification of the bulk organic matter is not achievable for Lake Bergsee based on the here presented bulk proxies (Fig. 2A to D).

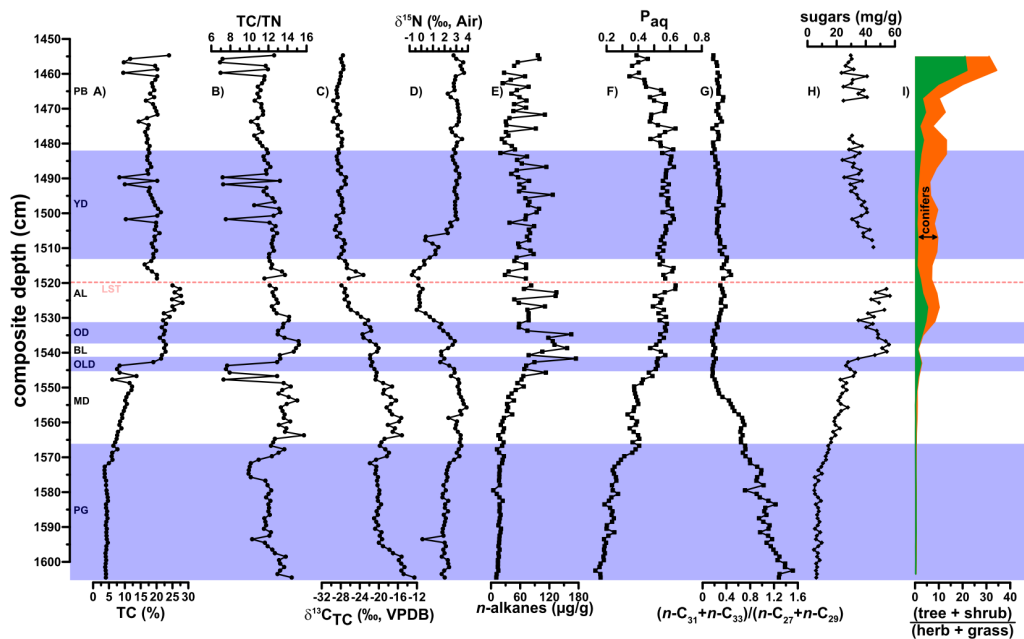


Fig. 2: Depth functions of Lake Bergsee. A) TC, B) TC/TN, C) $\delta^{13}C$, D) $\delta^{15}N$, E) total n -alkane concentration, F) P_{aq} (according to Ficken et al. 2000), G) n -alkane ratio $(n-C_{31}+n-C_{33})/(n-C_{27}+n-C_{29})$, H) total sugar concentration, and I) tree and shrub vs. herb and grass pollen ratio (green = sum of broadleaf trees and shrubs vs. herbs and grasses, orange = sum of broadleaf and coniferous trees and shrubs vs. herbs and grasses, orange minus green = coniferous proportion). Pollen results were taken from Duprat-Oualid et al. (2017). Background colors show time periods (according to Litt et al. 2001): PB = Preboreal, YD = Younger

227 Dryas, AL = Allerød, OD = Older Dryas, BL = Bølling, OLD = Oldest Dryas, MD = Meiendorf, PG = Pleniglacial.
228 LST = Laacher See Tephra.

229

230 3.2 Source identification of *n*-alkane and sugar biomarkers

231 The total *n*-alkane concentrations shown in Fig. 2E range between 4 and 175 µg/g and generally reflect the
232 TC depth profile. In order to assess aquatic vs. terrestrial *n*-alkane input P_{aq} values were calculated
233 according to Ficken et al. (2000): $P_{aq} = (n-C_{23} + n-C_{25}) / (n-C_{23} + n-C_{25} + n-C_{29} + n-C_{31})$. The P_{aq} index was
234 established to distinguish between submerged and floating vs. emergent and terrestrial plant *n*-alkane
235 input. The Lake Bergsee P_{aq} record shows values ranging from 0.1 to 0.6 (Fig. 4F). Below 1550 cm depth,
236 P_{aq} values < 0.4 suggest a predominant input of *n*-alkanes that derived from emergent plants. One might
237 be tempted to interpret the P_{aq} values of > 0.4 above 1550 cm depth in terms of a predominant input of
238 aquatic-derived *n*-alkanes. This is based on the finding that higher terrestrial plants produce long-chain *n*-
239 alkanes in order to build up the epicuticular wax layer (Eglinton and Hamilton 1967). By contrast, short-
240 chain *n*-alkanes are often associated with algae (Gelpi et al. 1970) and mid-chain *n*-alkanes are often
241 assumed to originate from submerged macrophytes (Ficken et al. 2000). However, note that Aichner et al.
242 (2018) recently stressed that P_{aq} is no robust proxy for aquatic influx in lake sediments from North Poland.
243 Rather, the sedimentary mid-chain *n*-alkanes *n*-C₂₃ and *n*-C₂₅ originate either from a mixture of aquatic and
244 terrestrial sources (during Allerød and the Younger Dryas onset) or are predominantly of terrestrial origin
245 (during Younger Dryas and Early Holocene). Given that e.g. *Betula* as one of the main pioneering species
246 during Late Glacial reforestation at Lake Bergsee (Duprat-Qualid et al. 2017) is well known to produce quite
247 considerable amounts of *n*-C₂₃ and *n*-C₂₅ (Tarasov et al. 2013; van den Bos et al. 2018), we recommend
248 caution when interpreting P_{aq} values from sedimentary archives. In the case of Lake Bergsee, we consider
249 the respective interpretation to be not robust. Moreover, litter degradation by soil microorganisms is
250 reported to cause changes of both mid-chain and long-chain *n*-alkanes patterns (Tu et al. 2011; Zech et al.
251 2011b). Hence, soil erosion from the catchment should be considered when interpreting P_{aq} values, too.

252 Long-chain *n*-alkanes are furthermore used in chemotaxonomic studies in order to distinguish between
253 different vegetation types. This is based on the observation that in grass and herbaceous plant material
254 the *n*-C₃₁ and *n*-C₃₃ homologues are often dominating, whereas trees and shrubs often show higher relative
255 concentrations of *n*-C₂₇ and *n*-C₂₉ (Maffei 1996; Zech et al. 2009). We used here the ratio $(n-C_{31}+n-C_{33})/(n-$
256 $C_{27}+n-C_{29})$, which range between 0.2 and 1.5 (Fig. 2G). This approach is comparable to the one of Schwark
257 et al. (2002), who used the *n*-C₂₇, *n*-C₂₉ and *n*-C₃₁ alkane distribution and pollen to reconstruct the Late

258 Glacial reforestation around Lake Steißlingen. While $(n\text{-}C_{31}+n\text{-}C_{33})/(n\text{-}C_{27}+n\text{-}C_{29})$ values > 1 can be
 259 interpreted in terms of enhanced input of grass-derived leaf material (below 1570 cm depth), values < 1
 260 suggest increased input of tree- and shrub-derived litter (above 1570 cm depth). The respective
 261 interpretation is overall in agreement with the pollen record of Lake Bergsee (Fig. 2I; Duprat-Oualid et al.
 262 2017) and corroborates that the investigated long-chain *n*-alkanes are primarily of terrestrial origin. Yet,
 263 there seems to be a time lag between the $(n\text{-}C_{31}+n\text{-}C_{33})/(n\text{-}C_{27}+n\text{-}C_{29})$ ratio and the pollen record. The *n*-
 264 alkane ratio declines most pronouncedly already during the Meiendorf Interstadial, whereas the tree and
 265 shrub pollen increase starts at the end of Meiendorf (compare Fig. 2G vs. I). Possibly, this minor
 266 discrepancy can be explained with shrub pollination rate having started with a delay compared to shrub
 267 spreading.

268 Total sugar concentrations for Lake Bergsee range between 4 and 57 mg/g (Fig. 2H) and reveal a highly
 269 significant correlation with TC ($r^2 = 0.88$, $p < 0.001$, $n = 128$). Recently, an approach was proposed for
 270 distinguishing between aquatic versus terrestrial sedimentary input based on the relative abundances of
 271 the sugar biomarkers ara, fuc and xyl (Hepp et al. 2016). Accordingly, $\text{fuc}/(\text{ara}+\text{xyl})$ ratios ranging between
 272 0.4 and 4.8 for the Lake Bergsee record are clearly indicative for a primarily aquatic origin of the sugars.
 273 Fig. 3 furthermore illustrates this interpretation in a ternary diagram where the relative abundances of
 274 ara, fuc and xyl for Lake Bergsee are compared with soil and sediment data from the literature according
 275 Hepp et al. (2016).

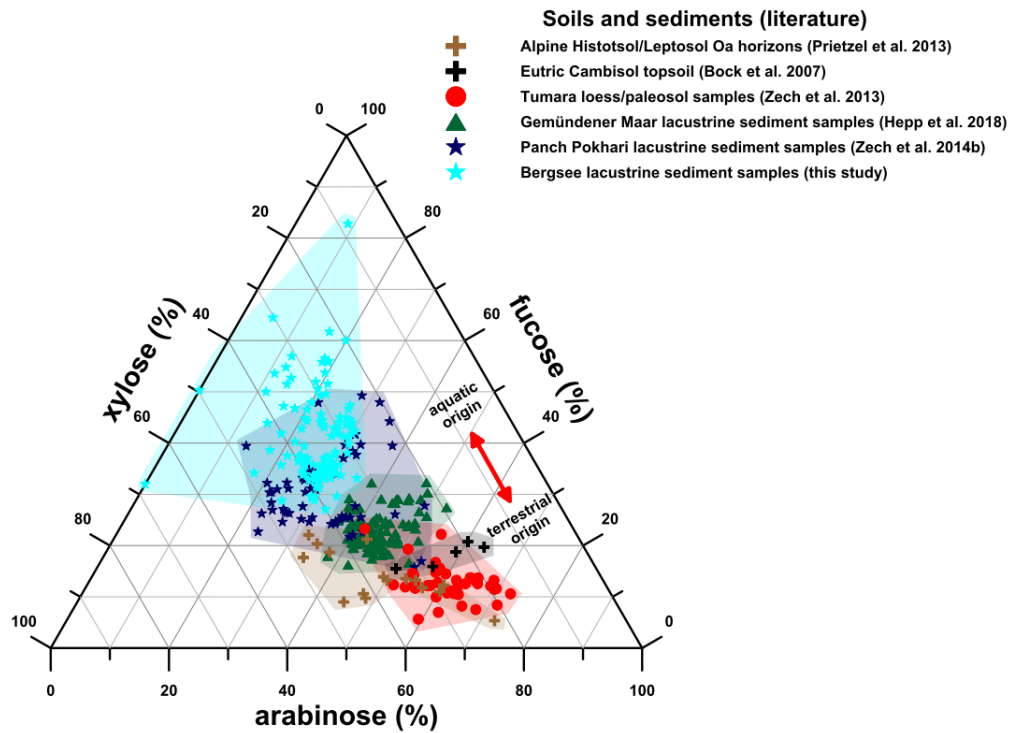


Fig. 3: Ternary diagram from Hepp et al. (2016) depicting the relative abundances of ara, xyl and fuc for Lake Bergsee sediment samples (this study) and comparative soil and sediment data compiled from the literature (Bock et al. 2007; Prietzel et al. 2013; Zech et al. 2013, 2014b; Hepp et al. 2018).

Summing up this subchapter, the long-chain *n*-alkanes *n*-C₂₇, *n*-C₂₉ and *n*-C₃₁ of the Lake Bergsee record are mainly of terrestrial origin, the sugar biomarkers ara, fuc and xyl are primarily of aquatic origin, and the mid-chain *n*-alkanes *n*-C₂₃ and *n*-C₂₅ in all likelihood represent a mixture of aquatic and terrestrial sources.

3.3 Lake Bergsee $\delta^2\text{H}_{n\text{-alkane}}$ record and its paleoclimatic interpretation

The $\delta^2\text{H}$ values of the investigated *n*-alkanes range between -215 to -173‰, -217 to -174‰, -222 to -138‰, -214 to -157‰ and -214 to -114‰ for *n*-C₃₁, *n*-C₂₉, *n*-C₂₇, *n*-C₂₅ and *n*-C₂₃, respectively (Fig. 4A). The *n*-C₂₉ and *n*-C₃₁ records show very similar $\delta^2\text{H}$ values. By contrast, *n*-C₂₇ yielded on average throughout the

record by +17‰ more positive $\delta^2\text{H}$ values. This could be explained with the different taxonomic origin of the alkane homologues. Leaf wax-derived long-chain *n*-alkanes are biosynthesized in the leaves of higher terrestrial plants, thus using mainly leaf water as hydrogen source, as e.g. reviewed by Sachse et al. (2012). Therefore, not only the plant source water is imprinted in terrestrial $\delta^2\text{H}_{n\text{-alkane}}$, moreover the leaf water evaporative enrichment plays an important role (Kahmen et al. 2013). As outlined above, grasses (e.g. *Poaceae*) are often characterized by highest relative abundances of *n*-C₃₁. At the same time, grass-derived *n*-alkanes (Fig. 2G) are known to be less sensitive recorders of leaf water enrichment compared to *n*-alkanes from other higher terrestrial plants (McInerney et al. 2011; Kahmen et al. 2013). The $\delta^2\text{H}$ offset between *n*-C₃₁ and *n*-C₂₇ is indeed largest during the Oldest Dryas and the early Older Dryas, when *Betula*, which is known to produce high amounts of *n*-C₂₇ (e.g. Tarasov et al. 2013) shows the highest pollen concentration (compare Fig. 4A and B) and *Poaceae* pollen concentration is decreasing (Duprat-Qualid et al. 2017).

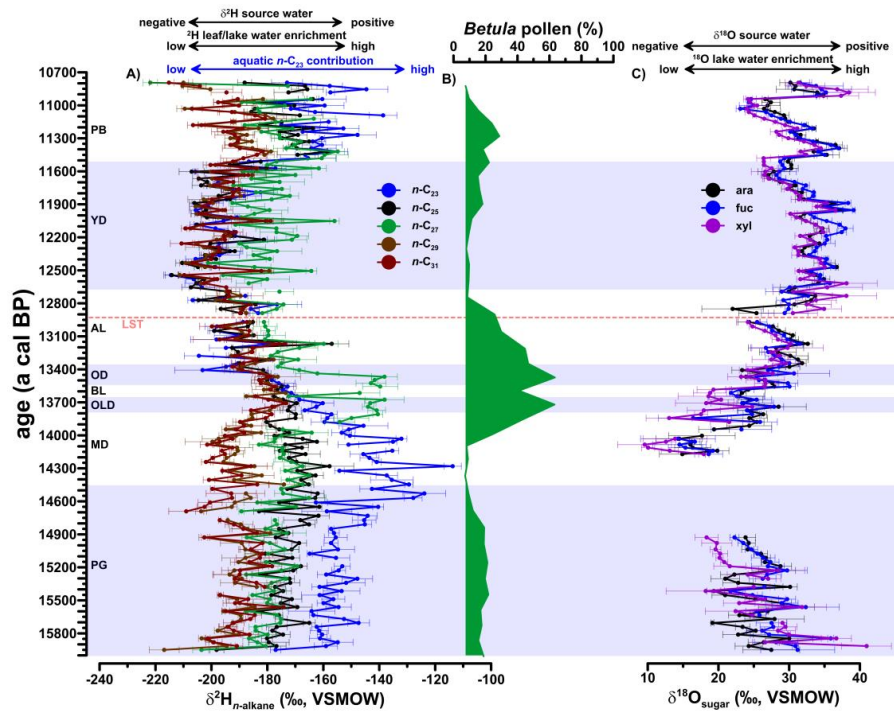


Fig. 4: A) $\delta^2\text{H}_{n\text{-alkane}}$ records (*n*-C₂₃, *n*-C₂₅, *n*-C₂₇, *n*-C₂₉ and *n*-C₃₁), B) *Betula* pollen record (Duprat-Qualid et al. 2017) and C) $\delta^{18}\text{O}_{\text{sugar}}$ records (ara, fuc, xyl) of Lake Bergsee. Background colors show time periods

305 according to Litt et al. (2001): PB = Preboreal, YD = Younger Dryas, AL = Allerød, OD = Older Dryas, BL =
306 Bølling, OLD = Oldest Dryas, MD = Meiendorf, PG = Pleniglacial. LST = Laacher See Tephra.

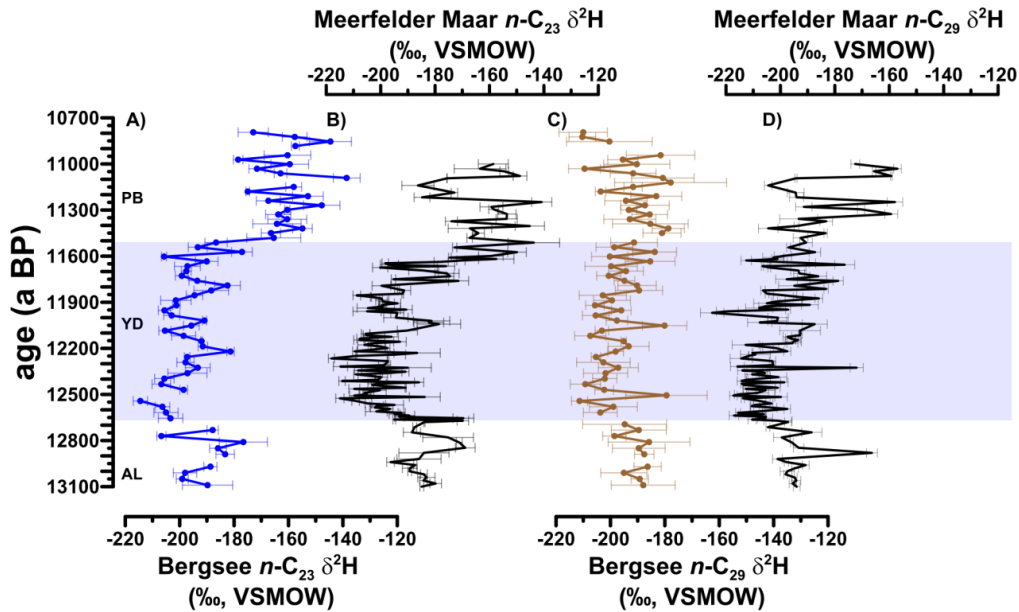
307

308 As outlined in section 3.2, $n\text{-C}_{23}$ in our Lake Bergsee record is most likely a mixture of terrestrial and aquatic
309 sources. The proposed partly aquatic origin of $n\text{-C}_{23}$ can help explaining the often more positive $\delta^2\text{H}$ values
310 of $n\text{-C}_{23}$ compared to the primarily terrestrial long-chain n -alkanes (Fig. 4A). While for long-chain n -alkanes
311 an biosynthetic fractionation factor (ϵ^2_{bio}) of -160‰ can be assumed (Sessions et al. 1999; Sachse et al.
312 2006), data from *Potamogeton* and surface sediments suggest an ϵ^2_{bio} fractionation factor of -82 to -88‰
313 for $n\text{-C}_{23}$ (Aichner et al. 2010). Accordingly, more positive $\delta^2\text{H}$ values of $n\text{-C}_{23}$ during the Pleniglacial,
314 Meiendorf and Preboreal of Lake Bergsee suggest a partly aquatic origin of $n\text{-C}_{23}$, whereas the absence of
315 an offset between $\delta^2\text{H}$ of $n\text{-C}_{23}$ and the long-chain n -alkanes during the Bølling, Older Dryas, Allerød and
316 Younger Dryas points to a primarily terrestrial origin of $n\text{-C}_{23}$. Therefore, the direct link between $\delta^2\text{H}$ values
317 of $n\text{-C}_{23}$ and $\delta^2\text{H}_{\text{lake-water}}$ (Aichner et al. 2010; Sachse et al. 2012) cannot be applied here.

318 In Fig. 5 the $n\text{-C}_{23}$ $\delta^2\text{H}$ record of Lake Bergsee is shown along with the $n\text{-C}_{23}$ $\delta^2\text{H}$ record from Lake
319 Meerfelder Maar (Rach et al. 2014) for comparison. Within age uncertainties, the $n\text{-C}_{23}$ $\delta^2\text{H}$ record of Lake
320 Bergsee resembles well the record from Lake Meerfelder Maar (Fig. 5A vs. B), which spans the time period
321 from 11,000 to 13,100 a varve BP (Rach et al. 2014). The $n\text{-C}_{23}$ $\delta^2\text{H}$ record from Lake Meerfelder Maar is
322 interpreted to reflect lake water and thus local precipitation. The lower $\delta^2\text{H}$ values of $n\text{-C}_{23}$ during the
323 Younger Dryas are therefore associated with lower regional air temperatures and by changes in $\delta^2\text{H}$ of the
324 moisture source associated with the freshwater input to the North Atlantic Ocean as well as with changes
325 in moisture source temperature and transport history (Rach et al. 2014). Importantly, for Lake Meerfelder
326 Maar the authors assumed the lake water enrichment was of minor importance. For Lake Bergsee,
327 however, lake water enrichment during drier periods is very likely, because the lake is characterized by
328 the absence of an inflowing creek (section 2.1). Fig. 5 also compares the $n\text{-C}_{29}$ $\delta^2\text{H}$ record of Lake Bergsee
329 with the $n\text{-C}_{29}$ $\delta^2\text{H}$ record from Lake Meerfelder Maar (Fig. 5C vs. D). Also these both records resemble
330 each other fairly well, except for larger fluctuations occurring in the Lake Meerfelder Maar record during
331 the Preboreal.

332 Summing up, the above outlined discussion reveals that the $\delta^2\text{H}_{n\text{-alkane}}$ record of Lake Bergsee cannot be
333 interpreted in a straightforward way. As illustrated in Fig. 4A, the main influencing factors which are
334 difficult to be disentangled and which cause $\delta^2\text{H}$ variations are: (i) $\delta^2\text{H}$ source water (\approx local precipitation)

335 changes, (ii) variable ^2H leaf/lake water enrichment, and (iii) variable contributions of aquatic versus
336 terrestrial $n\text{-C}_{23}$.



337
338 **Fig. 5:** Comparison between A) the $n\text{-C}_{23}$ $\delta^2\text{H}$ record of Lake Bergsee and B) the $n\text{-C}_{23}$ $\delta^2\text{H}$ record from Lake
339 Meerfelder Maar (Rach et al. 2014) and comparison between C) the $n\text{-C}_{29}$ $\delta^2\text{H}$ record of Lake Bergsee and
340 D) the $n\text{-C}_{29}$ $\delta^2\text{H}$ record from Lake Meerfelder Maar (Rach et al. 2014). Background colors show time
341 periods according to Litt et al. (2001): PB = Preboreal, YD = Younger Dryas, AL = Allerød, OD = Older Dryas,
342 BL = Bølling, OLD = Oldest Dryas, MD = Meiendorf, PG = Pleniglacial. LST = Laacher See Tephra.

344 **3.4 Lake Bergsee $\delta^{18}\text{O}_{\text{sugar}}$ record and its paleoclimatic interpretation**

345 The $\delta^{18}\text{O}$ values of ara, fuc and xyl range from +14.4 to +37.6‰, +14.3 to +39.1‰ and +9.6 to +40.9‰,
346 respectively, and resemble each other well (Fig. 4C). All three sugars are primarily of aquatic origin (see
347 discussion in section 3.2). Hence, the $\delta^{18}\text{O}_{\text{sugar}}$ record of Lake Bergsee can be interpreted in terms of
348 reflecting changes in (i) $\delta^{18}\text{O}$ source water (\approx local precipitation) and (ii) ^{18}O lake water enrichment (as
349 illustrated in Fig. 4C).

350 The lake water enrichment can be directly linked to relative humidity when the lake input to evaporation
351 ratio can be robustly defined (e.g. as terminal lake situation, input = evaporation; Gat 1971). This holds,

352 however, not valid for Lake Bergsee, due to the absence of a natural inflowing creek (see section 2.1).
353 Therefore, changes in the precipitation amount have to be taken into account, too (see section 3.2; Gibson
354 et al. 2016). Given that the $\delta^{18}\text{O}$ variability of precipitation (\approx source water) was unlikely larger than 8‰
355 during the Late Glacial-Holocene transition (von Grafenstein et al. 1998; Mayer and Schwark 1999), most
356 of the variability of the $\delta^{18}\text{O}_{\text{sugar}}$ record of Lake Bergsee can be attributed to variable lake water enrichment.
357 In order to highlight this, the $\delta^{18}\text{O}_{\text{sugar}}$ record of Lake Bergsee (calculated as weighted mean), is shown in
358 Fig. 6 in comparison to the $\delta^{18}\text{O}_{\text{carbonate}}$ record from Lake Steißlingen (Mayer and Schwark 1999) and the
359 stalagmite $\delta^{18}\text{O}_{\text{carbonate}}$ record from Hölloch Cave (Wurth et al. 2004). Those carbonate $\delta^{18}\text{O}$ records are
360 interpreted to reflect the local precipitation history. Lake Steißlingen is primarily fed by ground water via
361 submerged springs (Eusterhues et al. 2002) and the carbonate $\delta^{18}\text{O}$ values thus reflect mainly
362 precipitation. It is obvious that the $\delta^{18}\text{O}_{\text{sugar}}$ of Lake Bergsee shows opposite trends and a much higher
363 amplitude than the precipitation records (Fig. 8). The Younger Dryas-Preboreal transition reveals a shift of
364 around 5‰ towards more positive $\delta^{18}\text{O}_{\text{sugar}}$ values (Fig. 9A). This is in well in agreement with a
365 reconstructed $\delta^{18}\text{O}_{\text{lake-water}}$ shift of around 6‰ from Lake Gosciąz in Central Poland as inferred from the
366 sedimentary cellulose (Rozanski et al. 2010).

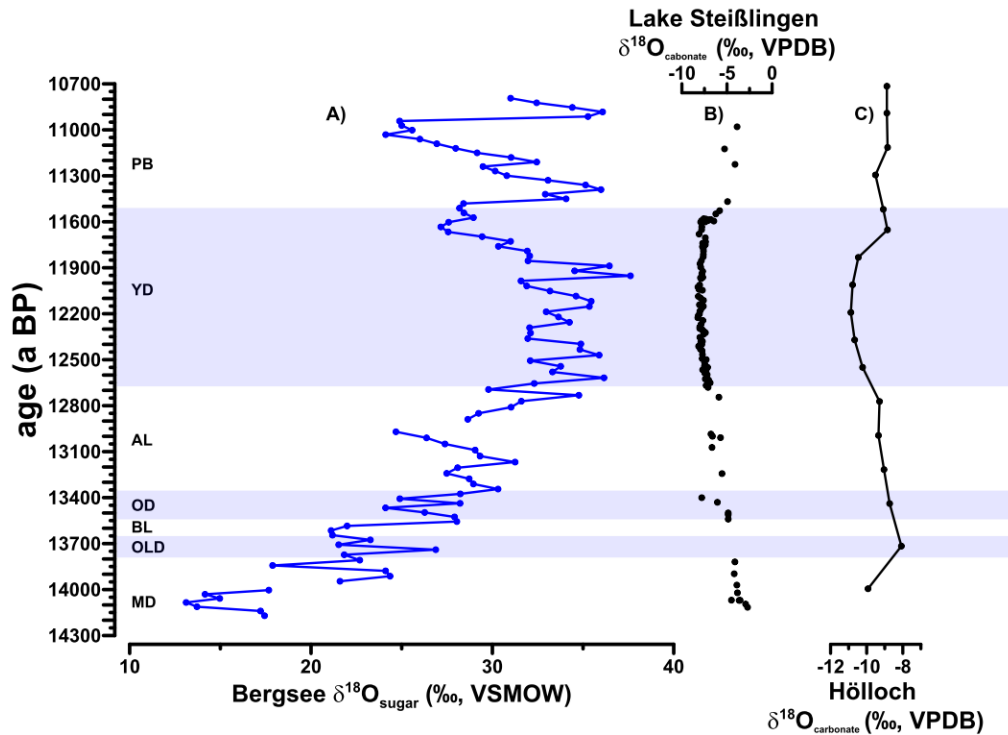


Fig. 6: Comparison between A) $\delta^{18}\text{O}_{\text{sugar}}$ record from Lake Bergsee and $\delta^{18}\text{O}_{\text{carbonate}}$ records from B) Lake Steißlingen (Mayer and Schwark 1999) and C) Hölloch cave stalagmite (Wurth et al. 2004). The $\delta^{18}\text{O}$ scale C is doubled compared to A and B due to a better visibility of the trends.

3.5 'Dual-biomarker approach' based on terrestrial versus aquatic $\delta^2\text{H}_{n\text{-alkane}}$ records – potential and limitations

The dual-biomarker approach was introduced by Rach et al. (2014, 2017) and uses the difference between terrestrial versus aquatic $\delta^2\text{H}_{n\text{-alkane}}$ values ($\epsilon_{\text{terrestrial-aquatic}}$). The basic assumption is that the long-chain n -alkanes such as $n\text{-C}_{29}$ are of terrestrial origin and reflect $\delta^2\text{H}$ of leaf water (precipitation altered by leaf water ^2H enrichment), whereas $n\text{-C}_{23}$ is of aquatic origin and reflects $\delta^2\text{H}$ of lake water that is not ^2H -enriched by evaporation (and thus reflects $\delta^2\text{H}$ of precipitation). When soil and lake water enrichment are negligible, such $\epsilon_{\text{terrestrial-aquatic}}$ values can be transferred into quantitative relative humidity values (Rach et al. 2017). Given that neither soil nor lake water ^2H enrichment can be excluded for every study site, this represents one potential uncertainty of the dual-biomarker approach (Fig. 7). Especially lake water enrichment cannot be ruled out under dry and/or warm climatic conditions (see Fig. 8 in Hou et al. 2008). When lake water is/was affected by ^2H enrichment in the past, the $\epsilon_{\text{terrestrial-aquatic}}$ values are no longer a

robust proxy for leaf water evapotranspirative enrichment. Another uncertainty concerns the hydrogen fractionation during *n*-alkane biosynthesis (ϵ^2_{bio}) (Fig. 7). There is increasing evidence that this fractionation is not constant between different species/organisms (e.g. review from Pedentchouk and Zhou, 2018). This holds especially true for aquatic ϵ^2_{bio} values. While Muschitiello et al. (2015) and Rach et al. (2014) assume that aquatic ϵ^2_{bio} is smaller than terrestrial ϵ^2_{bio} , Sachse et al. (2006), Jacob et al. (2007) and Seki et al. (2011) set aquatic ϵ^2_{bio} equal to terrestrial ϵ^2_{bio} and Hou et al. (2008) show a case in which aquatic ϵ^2_{bio} is larger than terrestrial ϵ^2_{bio} . Please note, that Jacob et al. (2007) called their difference between the terrestrial $\delta^2\text{H}_{n\text{-alkane}}$ (C_{31}) and aquatic $\delta^2\text{H}_{n\text{-alkanoic}}$ (C_{16}) acid $\alpha_{\text{TA/wat}}$. They furthermore only mention the fractionation factor between $\delta^2\text{H}_{n\text{-alkanoic}}$ (C_{16}) and source (lake) water to be -170‰. However, the ϵ^2_{bio} between terrestrial $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^2\text{H}_{\text{leaf-water}}$ can be assumed to be close to that value, which is only slightly larger than the commonly assumed -160‰ for terrestrial *n*-alkanes (see section 3.3; Sessions et al. 1999; Freimuth et al. 2017). Finally, the paradigmatic source identification of the *n*-alkanes (long-chain *n*-alkanes originate from terrestrial plants whereas mid-chain *n*-alkanes such as *n*- C_{23} originate from aquatic organisms) may not always hold true (see sections 3.2 and 3.3). As mentioned above, also Aichner et al. (2018) recently emphasized that *n*- C_{23} in a lacustrine sedimentary record from Poland is either completely or at least partly of terrestrial origin. Given the above-discussed uncertainties, particularly including our interpretation that *n*- C_{23} in Lake Bergsee is a mixture of aquatic and terrestrial sources, we consider the dual-biomarker approach to be not robustly applicable to our $\delta^2\text{H}_{n\text{-alkane}}$ record and refrain from a respective application.

3.6 'Coupled $\delta^2\text{H}_{n\text{-alkane}}$ - $\delta^{18}\text{O}_{\text{sugar}}$ approach' – potential and limitations

When applying the coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach the following assumptions have to be made: (i) lake/leaf water $\delta^2\text{H}/\delta^{18}\text{O}$ values can be reconstructed from $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ values by applying constant biosynthetic fractionation factors. (ii) Lake/leaf water evapo(transpi)rative enrichment occur

419 along a local evaporation line, and the slope for such a line can be derived from a simplified Craig-Gordon
 420 model. (iii) The $\delta^2\text{H}/\delta^{18}\text{O}$ values of plant/lake source water reflect precipitation, which typically plots along
 421 a well-defined meteoric water line. The intersect between the local evaporation line throughout an
 422 individual leaf/lake water point and the meteoric water thus allow the reconstruction of $\delta^2\text{H}/\delta^{18}\text{O}_{\text{source water}}$
 423 ($\approx \delta^2\text{H}/\delta^{18}\text{O}_{\text{precipitation}}$). As such, the coupled $\delta^2\text{H}-\delta^{18}\text{O}$ approach is a promising tool to disentangle between
 424 the leaf/lake water evapo(transpi)rative enrichment and the precipitation signal incorporated in the
 425 biomarkers.

426 In the case of terrestrial biomarkers, the reconstructed $\delta^2\text{H}/\delta^{18}\text{O}_{\text{leaf-water}}$ can be used for calculating
 427 deuterium-excess of leaf water. These deuterium-excess values can be converted into quantitative relative
 428 humidity values. When aquatic biomarkers are used to reconstruct $\delta^2\text{H}/\delta^{18}\text{O}_{\text{lake-water}}$, those values allow
 429 reconstructing the deuterium-excess of lake water. Those values can be used as lake water
 430 evaporation/desiccation proxy. It should be noted that the deuterium-excess calculations follow the
 431 formulation introduced by Dansgaard (1964). The coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ approach was successfully
 432 applied to terrestrial sedimentary archives in order to derive relative humidity history (Zech et al. 2013;
 433 Hepp et al. 2017). It was furthermore validated using a climate transect study by Tuthorn et al. (2015),
 434 who called the $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ coupling a ‘paleohygrometer approach’. At the same time, Hepp et al.
 435 (2015) realized the adoption of the coupled approach to a lacustrine sedimentary archive. Based on
 436 aquatic biomarkers the authors reconstructed lake water evaporation history along with $\delta^2\text{H}/\delta^{18}\text{O}_{\text{precipitation}}$.

437 Apart from these potentials, the coupled $\delta^2\text{H}_{n\text{-alkane}}-\delta^{18}\text{O}_{\text{sugar}}$ approach has also limitations/uncertainties
 438 (Fig. 8): (i) A clear source identification of the biomarkers is needed. (ii) The ϵ^2_{bio} and the oxygen
 439 fractionation during sugar biosynthesis ($\epsilon^{18}_{\text{bio}}$) are assumed to be robust and constant. For a robust
 440 deuterium-excess of leaf water interpretation also the soil water enrichment has to be ruled out.
 441 Moreover, the slope of the local evaporation line has to be approximated. While for leaf water this seems
 442 to be a minor issue due to rather small variations (Allison et al. 1985; Walker and Brunel 1990; Bariac et
 443 al. 1994; Mayr 2002), the lake water local evaporation line seem to be much more variable (Gibson et al.
 444 2008). This was taken into consideration by Hepp et al. (2015) by making reconstructions with different
 445 slopes.

446 In the case of Lake Bergsee, we refrain from applying the coupled $\delta^2\text{H}-\delta^{18}\text{O}$ approach primarily because
 447 we have neither (i) a reliable pure aquatic coupled $\delta^2\text{H}$ and $\delta^{18}\text{O}$ record (ii) nor a reliable pure terrestrial
 448 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ record.

471 During the last years, two concepts were developed in order to disentangle the precipitation from the
472 evaporation signal and to reconstruct relative humidity: the ‘dual-biomarker approach’ by Rach et al.
473 (2014) and the ‘coupled $\delta^2\text{H}_{n\text{-alkane}}\text{-}\delta^{18}\text{O}_{\text{sugar}}$ approach’ by Zech et al. (2013) and Hepp et al. (2015). Despite
474 the great potential of both approaches, we advise caution not to over-interpret sedimentary $\delta^2\text{H}_{n\text{-alkane}}$ and
475 $\delta^{18}\text{O}_{\text{sugar}}$ records. Fundamental assumptions underlying the two approaches may not be robust in every
476 case study. This concerns primarily:

- 477 • Lake water enrichment, which is assumed to be negligible in the dual-biomarker approach.
- 478 • Source identification of the biomarkers: In the case of Lake Bergsee, $n\text{-C}_{23}$ is in all likelihood a
479 mixture of aquatic and terrestrial sources and thus does not reflect $\delta^2\text{H}$ of lake water.
- 480 • Biosynthetic fractionation factors: there seems to be a large offset between terrestrial versus
481 aquatic ϵ^2_{bio} values (around -160 versus around -85‰). Hence, minor changes in the contribution
482 of terrestrial versus aquatic $n\text{-alkanes}$ are likely to have a large impact on the $\delta^2\text{H}$ record of $n\text{-C}_{23}$.

483 These state-of-the-art uncertainties clearly limit the robustness of both approaches and hindered their
484 application to the Lake Bergsee $\delta^2\text{H}_{n\text{-alkane}}$ and $\delta^{18}\text{O}_{\text{sugar}}$ records.

485

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698

Additional publications

A list of additional publications not included in the cumulative PhD thesis is given below:

Johannes Hepp, Mario Tuthorn, Roland Zech, Ines Mügler, Frank Schlütz, Wolfgang Zech, Michael Zech (2015): Reconstructing lake evaporation history and the isotopic composition of precipitation by a coupled $\delta^{18}\text{O}$ - $\delta^2\text{H}$ biomarker approach. *Journal of Hydrology*, 529:622-631, <https://doi.org/10.1016/j.jhydrol.2014.10.012>.

Martin Bachmann, **Johannes Hepp**, Michael Zech, Michael Bulang, Annette Zeyner (2017): Application of natural wax markers in equine nutrition studies – current state, limitations and perspectives, *Livestock Science*, 208:77-89, <https://doi.org/10.1016/j.livsci.2017.12.010>.

Katja Osterloh, Nadine Tauchnitz, Oliver Spott, **Johannes Hepp**, Sabine Bernsdorf, Ralph Meissner (2018): Changes of methane and nitrous oxide emissions in a transition bog in central Germany (German National Park Harz Mountains) after rewetting, *Wetlands Ecology and Management*, 26:87-102, <https://doi.org/10.1007/s11273-017-9555-x>.

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